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OF THE  
TORREY BOTANICAL CLUB  
JANUARY 1926

An ecological study of certain ferns: *Pellaea atropurpurea* (L.)  
Link and *Pellaea glabella* Mettenius<sup>1</sup>

F. L. PICKETT AND MILDRED E. MANUEL

(WITH FOUR FIGURES)

Following similar work with other xerophytic ferns, and in conjunction with morphological studies of the two species of *Pellaea* here considered, an experimental study of certain adaptive characters has been made. These two species of ferns are generally well known as inhabitants of locations which are, at least in some seasons, subject to severe drought and other desiccating agencies. Various suggestions have been made at times in possible explanations of the ability of these ferns to withstand difficult growth conditions. These have been summarized by Miss Hayes<sup>2</sup> and need not be reviewed here. Up to this time, however, no experimental work seems to have been done touching the importance of the gametophyte generation in this relation.

The studies reported here were made with cultures of *P. atropurpurea* and *P. glabella* grown from spores collected in Monroe County, Indiana, in 1923, upon soil taken from the same locality. The prothallia were allowed to develop to maturity through a period of 15 to 20 weeks and then were subjected to various desiccating conditions. The results are given in brief form below.

A. Cultures were allowed to become dry in the greenhouse where the atmosphere showed about 20 per cent moisture.

On Jan. 19, 1924, cultures in the greenhouse were allowed to become air dry, and remained in that condition up to May 26, (124 days) when small portions were tested and showed nearly

<sup>1</sup> Contribution No. 4 from the Department of Botany of the State College of Washington.

<sup>2</sup> D. W. Hayes. Some studies of apogamy in *Pellaea atropurpurea* (L.) Link. Trans. Am. Micro. Soc. 43: 119-135. July, 1924.

[The BULLETIN for December (52: 491-553) was issued 6 January 1926.]

all plants living. After thorough wetting on this date these cultures were allowed to dry again and were placed in a cool, dry cupboard on June 5, where they remained untouched up to October 25 (142 days). These cultures had been exposed to dry air, and had received no water supply through this period of 142 days, and when they were watered showed more than half of the plants living. The other cultures were allowed to remain dry in the greenhouse from Oct. 25, 1924 to March 24, 1925 (150 days) and when watered showed fully three-fourths of the plants living. One of these cultures had been wholly dry since June 5, a total period of nine months and twenty days. The plants in these cultures were mature, many showing sporophytes with two or more leaves. The ability to survive natural drought conditions is seemingly the same for both prothallia and sporophytes.

B. Plants with a thin layer of attached soil which had been allowed to dry as in A, were placed in closed desiccators over anhydrous calcium chloride.

Portions of cultures which had been dry in the greenhouse since Jan. 19, 1924, were on May 26 placed in large closed desiccators. On Feb. 24, 1925, parts of this material were put under conditions for recovery with the result that a few prothallia of *P. atropurpurea* and many of *P. glabella* showed survival of cell masses sufficient for regeneration as noted later. The ability to survive these extreme conditions seems in no way connected with size or stage of development of prothallia; the smallest as well as large, embryo-bearing prothallia survived in equal numbers. The sporophytes, however, show higher percentage of survival than the prothallia.

At this time, Feb. 24, 1925, portions of the cultures, soil and plants, were removed from the desiccators, weighed, and after drying in an oven for five hours at a temperature of from 90° to 110° C., they were weighed again. The loss of weight was as follows:

For *P. atropurpurea* .00043 gm. per gm. or .043% of material weighed.

For *P. glabella* .00058 gm. per gm. or .058% of material weighed.

No explanation can be offered for the difference in loss by the two samples, unless there was a difference in the relative

amount of plant and soil materials in the two. In any case the amount of water, suggested by the slight loss indicated, is so far below that usually found in living plant tissues, or in soil where living plants are found, as to be worthy of note.

How long these fern prothallia and young sporophytes may be subjected to such extreme conditions and survive is still a question. A final test is being completed as this report is being written, Aug. 30, 1925. The portions of cultures placed in the desiccators on May 26, 1924, after being dry in the greenhouse for 138 days, were put under conditions for growth on July 28, 1925, a total of  $18\frac{1}{2}$  months with results as follows. After the plants and soil had been moistened most of the plants soon took on the dark color characteristic of dead tissue; but in a few plants small areas of tissue retained the bright green color of living plants. Now, one month after growth conditions had been provided, small masses of cells, evidently secondary prothallia, have appeared as outgrowths from these green areas.

For the benefit of those who may be interested somewhat in the technique of this work, the following details should perhaps be given. The fern room in the greenhouse where this work has been carried on has a south exposure. It is used entirely for this and similar drought experiments. Through the winter months its temperature is maintained at very nearly  $21^{\circ}$  C. ( $70^{\circ}$  F.), with rare drops to  $10^{\circ}$  C. and rises to  $27^{\circ}$  C. In summer the ventilators are opened, but the temperature occasionally reaches  $35^{\circ}$  C. The average humidity is somewhat below 20 percent except during periods of actual rain or fog.

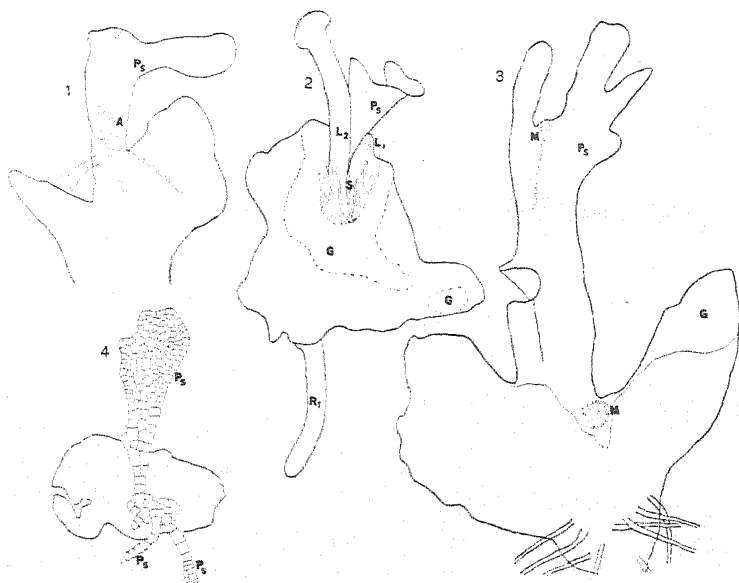
In determining whether or not plants are alive after periods of desiccation, the soil has been moistened, without flooding the plants; and observations made over such period, from two to five weeks, as needed to see *actual growth* before a report of "living plants" has been made.

As described elsewhere, these two ferns are wholly apogamous, showing no evidence of archegonial growth, but producing sporophytes through the increased activity and differentiation of masses of prothallial cells. The production of embryos seems not hindered by the desiccation experiments described. In many cases the appearance of embryo sporophytes has been the first sure sign of growth of surviving plants. Normal antheridia are produced frequently, and the formation within these of

spermatozoids, that may be extruded and then swim about freely in water within a few days after the plants have been returned to growth conditions, is, of course, evidence of life.

#### PROTHALLIAL PROLIFERATION

Old prothallia of *P. atropurpurea* show a tendency to produce secondary prothallia through proliferation. Marginal pro-



FIGS. 1-4. Prothallial proliferation: FIGS. 1-3, *Pellaea atropurpurea*; FIG. 4, *P. glabella*. Of special interest are the antheridia, A, on a secondary prothallium, Ps, in FIG. 1, the secondary prothallium produced after an embryo has started in FIG. 2, the beginning of an embryo on a secondary prothallium in FIG. 3, and the beginning of several prothallia from a small growing area in FIG. 4. All  $\times 8$ . G, growing area; L, leaf; R, root; M, very young embryo; Ps, secondary prothallium; S, stem.

jections showing broadened apex and narrow base similar to normal prothallia are produced either from cells near the meristematic group at the base of the sinus or from other isolated marginal groups (FIGS. 1, 2, 3). Examination of a culture that had come into growth again after a long period of desiccation showed, together with many apogamous embryos, numerous secondary prothallia as just described. In this culture, however, this proliferation was nearly wholly connected with the meristematic tissue at the base of the sinus. In most cases these ferns



show such proliferation from tissue near the base of the sinus rather than from many marginal groups, as in *Camptosorus rhizophyllus*.<sup>3</sup>

Similar activity has been observed in prothallia of *P. glabella*, although the origin of secondary prothallia is different. Prothallia, which had been subjected to extreme desiccation, have in some cases shown only small areas of living cells after being returned to growing conditions. From these small living areas, miscellaneous scattered over the prothallium, new growth has produced secondary prothallia similar to those noted above (FIG. 4).

That these proliferations should be considered as true secondary prothallia is shown by the fact that they have been observed with antheridia as on other prothallia, and with apogamous embryos (FIG. 3).

Three items in history of the gametophyte of these ferns seem to have direct bearing upon their distribution: the ability to survive extreme drought, the power of renewed growth shown by the production of secondary prothallia, and the production of sporophytes apogamously.

The seasonal development may be given as follows:—

Fertile fronds appear in March or April and show mature spores by the first of July. When the atmosphere is dry the sporangia and spores are closely inclosed by the inrolling of the marginal false indusium. In moist periods the spores are freed, and come in contact with the nearby soil or cliff face. If the moisture persists for several days, the spores germinate. Alternate periods of abundant moisture and of drought, such as are common throughout the ranges of these ferns through the late summer, merely serve to arrest the growth of the prothallia. Free from the need of water as an aid in bringing about fertilization, when they have reached a proper stage of maturity, the prothallia produce embryo sporophytes apogamously, and these in three or four years produce spores.

PULLMAN, WASHINGTON

<sup>3</sup> Pickett, F. L. Some ecological adaptations of certain fern prothallia *Camptosorus rhizophyllus* Link., *Asplenium platyneuron* Oakes. Am. Jour. Bot. 1: 477-498. f. 1-19 + pl. 49, 50. 1914.



## The individualistic concept of the plant association\*

H. A. GLEASON

The continued activity of European ecologists, and to a somewhat smaller extent of American ecologists as well, in discussing the fundamental nature, structure, and classification of plant associations, and their apparently chronic inability to come to any general agreement on these matters, make it evident that the last word has not yet been said on the subject. Indeed, the constant disagreement of ecologists, the readiness with which flaws are found by one in the proposals of another, and the wide range of opinions which have been ably presented by careful observers, lead one to the suspicion that possibly many of them are somewhat mistaken in their concepts, or are attacking the problem from the wrong angle.

It is not proposed to cite any of the extensive recent literature on these general subjects, since it is well known to all working ecologists. Neither is it necessary to single out particular contributions for special criticism, nor to point out what may appear to us as errors in methods or conclusions.

It is a fact, as Dr. W. S. Cooper has brought out so clearly in a manuscript which he has allowed me to read, and which will doubtless be in print before this, that the tendency of the human species is to crystallize and to classify his knowledge; to arrange it in pigeon-holes, if I may borrow Dr. Cooper's metaphor. As accumulation of knowledge continues, we eventually find facts that will not fit properly into any established pigeon-hole. This should at once be the sign that possibly our original arrangement of pigeon-holes was insufficient and should lead us to a careful examination of our accumulated data. Then we may conclude that we would better demolish our whole system of arrangement and classification and start anew with hope of better success.

Is it not possible that the study of synecology suffers at the present time from this sort of trouble? Is it not conceivable that, as the study of plant associations has progressed from its originally simple condition into its present highly organized and complex state, we have attempted to arrange all our facts in ac-

\* Contributions from The New York Botanical Garden, No. 279.

cordance with older ideas, and have come as a result into a tangle of conflicting ideas and theories?

No one can doubt for a moment that there is a solid basis of fact on which to build our study of synecology, or that the study is well worth building. It is the duty of the botanist to translate into intelligible words the various phenomena of plant life, and there are few phenomena more apparent than those of their spatial relations. Plant associations exist; we can walk over them, we can measure their extent, we can describe their structure in terms of their component species, we can correlate them with their environment, we can frequently discover their past history and make inferences about their future. For more than a century a general progress in these features of synecology can be traced.

It has been, and still is, the duty of the plant ecologist to furnish clear and accurate descriptions of these plant communities, so that by them the nature of the world's vegetation may be understood. Whether such a description places its emphasis chiefly on the general appearance of the association, on a list of its component species, on its broader successional relations, or on its gross environment, or whether it enters into far greater detail by use of the quadrat method, statistical analysis,<sup>1</sup> or exact environometry, it nevertheless contributes in every case to the advancement of our understanding of each association in detail and of vegetation in all its aspects in general.

It is only natural that we should tend to depart from the various conclusions which we have reached by direct observation or experiment, and to attempt other more general deductions as well. So we invent special terms and methods for indicating the differences between associations and the variation of the plant life within a single community. We draw conclusions for ourselves, and attempt to lay down rules for others as to ways and means of defining single associations, by character species, by statistical studies, by environmental relations, or by successional history. We attempt to classify associations, as individual

<sup>1</sup> Pavillard has cast serious doubt on the efficiency of the statistical method in answering questions of synecology. His argument, based solely on European conditions, needs of course no reply from America, but it may properly be pointed out that the intimate knowledge of vegetational structure obtained in this way may easily lead to a much fuller appreciation of synecological structure, entirely aside from any merits of the actual statistical results.

examples of vegetation, into broader groups, again basing our methods on various observable features and arriving accordingly at various results. We even enter the domain of philosophy, and speculate on the fundamental nature of the association, regard it as the basic unit of vegetation, call it an organism, and compare different areas of the same sort of vegetation to a species.

The numerous conclusions in synecology which depend directly upon observation or experiment are in the vast majority of cases entirely dependable. Ecologists are trained to be accurate in their observations, and it is highly improbable that any have erred purposely in order to substantiate a conclusion not entirely supported by facts. But our various theories on the fundamental nature, definition, and classification of associations extend largely beyond the bounds of experiment and observation and represent merely abstract extrapolations of the ecologist's mind. They are not based on a pure and rigid logic, and suffer regularly from the vagaries and errors of human reason. A geneticist can base a whole system of evolution on his observations of a single species: ecologists are certainly equally gifted with imagination, and their theories are prone to surpass by far the extent warranted by observation.

Let us then throw aside for the moment all our pre-conceived ideas as to the definition, fundamental nature, structure, and classification of plant associations, and examine step by step some of the various facts pertinent to the subject which we actually know. It will not be necessary to illustrate them by reference to definite vegetational conditions, although a few instances will be cited merely to make our meaning clear. Other illustrations will doubtless occur to every reader from his own field experience.

We all readily grant that there are areas of vegetation, having a measurable extent, in each of which there is a high degree of structural uniformity throughout, so that any two small portions of one of them look reasonably alike. Such an area is a plant association, but different ecologists may disagree on a number of matters connected with such an apparently simple condition. More careful examination of one of these areas, especially when conducted by some statistical method, will show that the uniformity is only a matter of degree, and that two sample quadrats with precisely the same structure can scarcely be discovered.

Consequently an area of vegetation which one ecologist regards as a single association may by another be considered as a mosaic or mixture of several, depending on their individual differences in definition. Some of these variations in structure (if one takes the broader view of the association) or smaller associations (if one prefers the narrower view) may be correlated with differences in the environment. For example, the lichens on a tree-trunk enjoy a different environment from the adjacent herbs growing in the forest floor. A prostrate decaying log is covered with herbs which differ from the ground flora in species or in relative numbers of individuals of each species. A shallow depression in the forest, occupied by the same species of trees as the surroundings, may support several species of moisture-loving herbs in the lower stratum of vegetation. In other cases, the variations in vegetational structure may show no relation whatever to the environment, as in the case of a dense patch of some species which spreads by rhizomes and accordingly comes to dominate its own small area. The essential point is that precise structural uniformity<sup>2</sup> of vegetation does not exist, and that we have no general agreement of opinion as to how much variation may be permitted within the scope of a single association.

In our attempts to define the limits of the association, we have but two actually observable features which may be used as a basis, the environment and the vegetation. Logically enough, most ecologists prefer the latter, and have developed a system based on character-species. In northern latitudes, and particularly in glaciated regions, where most of this work has been done, there is a wide diversity in environment and a comparatively limited number of species in the flora. A single association is therefore occupied by few species, with large numbers of individuals of each, and it has not been difficult to select from most associations a set of species which are not only fairly common and abundant, but which are strictly limited to the one association. But in many parts of the tropics, where diversity of environment has been reduced to a minimum by the practical completion of most physiographic processes and by the long-continued cumulation of plant reactions, and where the flora is

<sup>2</sup> It has often occurred to the writer that much of the structural variation in an association would disappear if those taxonomic units which have the same vegetational form and behavior could be considered as a single ecological unit.

extraordinarily rich in species, such a procedure is impracticable or even impossible. Where a single hectare may contain a hundred species of trees, not one of which can be found in an adjacent hectare, where a hundred quadrats may never exhibit the same herbaceous species twice, it is obvious that the method of characteristic species is difficult or impracticable.

It is also apparent that different areas of what are generally called the same association do not always have precisely the same environment. A grove of *Pinus Strobus* on soil formed from decomposed rocks in the eastern states, a second on the loose glacial sands of northern Michigan, and a third on the sandstone cliffs of northern Illinois are certainly subject to different environmental conditions of soil. An association of prairie grass in Illinois and another in Nebraska undoubtedly have considerable differences in rainfall and available water. A cypress swamp in Indiana has a different temperature environment from one in Florida.

Two environments which are identical in regard to physiography and climate may be occupied by entirely different associations. It is perfectly possible to duplicate environments in the Andes of southern Chile and in the Cascade Mountains of Oregon, yet the plant life is entirely different. Duplicate environments may be found in the deserts of Australia and of Arizona, and again have an entirely different assemblage of species. Alpine summits have essentially the same environment at equal altitudes and latitudes throughout the world, apart from local variations in the component rock, and again have different floras. It seems apparent, then, that environment can not be used as a means of defining associations with any better success than the vegetation.

At the margin of an association, it comes in contact with another, and there is a transition line or belt between them. In many instances, particularly where there is an abrupt change in the environment, this transition line is very narrow and sharply defined, so that a single step may sometimes be sufficient to take the observer from one into the other. In other places, especially where there is a very gradual transition in the environment, there is a correspondingly wide transition in the vegetation. Examples of the latter condition are easily found in any arid mountain region. The oak forests of the southern Coast Range

in California in many places descend upon the grass-covered foothills by a wide transition zone in which the trees become very gradually fewer and farther apart until they ultimately disappear completely. In Utah, it may be miles from the association of desert shrubs on the lower elevations across a mixture of shrubs and juniper before the pure stands of juniper are reached on the higher altitudes. It is obvious, therefore, that it is not always possible to define with accuracy the geographical boundaries of an association and that actual mixtures of associations occur.

Such transition zones, whether broad or narrow, are usually populated by species of the two associations concerned, but instances are not lacking of situations in which a number of species seem to colonize in the transition zone more freely than in either of the contiguous associations. Such is the case along the contact between prairie and forest, where many species of this type occur, probably because their optimum light requirements are better satisfied in the thin shade of the forest border than in the full sun of the prairie or the dense shade of the forest. Measured by component species such a transition zone rises almost to the dignity of an independent association.

Species of plants usually associated by an ecologist with a particular plant community are frequently found within many other types of vegetation. A single boulder, partly exposed above the ground at the foot of the Rocky Mountains in Colorado, in the short-grass prairie association, may be marked by a single plant of the mountain shrub *Cercocarpus*. In northern Michigan, scattered plants of the moisture-loving *Viburnum cassinoides* occur in the xerophytic upland thickets of birch and aspen. Every ecologist has seen these fragmentary associations, or instances of sporadic distribution, but they are generally passed by as negligible exceptions to what is considered a general rule.

There are always variations in vegetational structure from year to year within every plant association. This is exclusive of mere periodic variations from season to season, or aspects, caused by the periodicity of the component species. Slight differences in temperature or rainfall or other environmental factors may cause certain species to increase or decrease conspicuously in number of individuals, or others to vary in their vigor or luxuriance. Coville describes, in this connection, the remarkable variation in size of an *Amaranthus* in the Death Valley, which was



three meters high in a year of abundant rainfall, and its progeny only a decimeter high in the following year of drought.

The duration of an association is in general limited. Sooner or later each plant community gives way to a different type of vegetation, constituting the phenomenon known as succession. The existence of an association may be short or long, just as its superficial extent may be great or small. And just as it is often difficult and sometimes impossible to locate satisfactorily the boundaries of an association in space, so is it frequently impossible to distinguish accurately the beginning or the end of an association in time. It is only at the center of the association, both geographical and historical, that its distinctive character is easily recognizable. Fortunately for ecology, it commonly happens that associations of long duration are also wide in extent. But there are others, mostly following fires or other unusual disturbances of the original vegetation, whose existence is so limited, whose disappearance follows so closely on their origin, that they scarcely seem to reach at any time a condition of stable equilibrium, and their treatment in any ecological study is difficult. The short-lived communities bear somewhat the same relation to time-distribution as the fragmentary associations bear to space-distribution. If our ecological terminology were not already nearly saturated, they might be termed ephemeral associations.

Now, when all these features of the plant community are considered, it seems that we are treading upon rather dangerous ground when we define an association as an area of uniform vegetation, or, in fact, when we attempt any definition of it. A community is frequently so heterogeneous as to lead observers to conflicting ideas as to its associational identity, its boundaries may be so poorly marked that they can not be located with any degree of accuracy, its origin and disappearance may be so gradual that its time-boundaries can not be located; small fragments of associations with only a small proportion of their normal components of species are often observed; the duration of a community may be so short that it fails to show a period of equilibrium in its structure.

A great deal has been said of the repetition of associations on different stations over a considerable area. This phenomenon is striking, indeed, and upon it depend our numerous attempts to

classify associations into larger groups. In a region of numerous glacial lakes, as in parts of our northeastern states, we find lake after lake surrounded by apparently the same communities, each of them with essentially the same array of species in about the same numerical proportions. If an ecologist had crossed Illinois from east to west prior to civilization, he would have found each stream bordered by the same types of forest, various species of oaks and hickories on the upland, and ash, maple, and sycamore in the alluvial soil nearer the water. But even this idea, if carried too far afield, is found to be far from universal. If our study of glacial lakes is extended to a long series, stretching from Maine past the Great Lakes and far west into Saskatchewan, a very gradual but nevertheless apparent geographical diversity becomes evident, so that the westernmost and easternmost members of the series, while still containing some species in common, are so different floristically that they would scarcely be regarded as members of the same association. If one examines the forests of the alluvial floodplain of the Mississippi River in southeastern Minnesota, that of one mile seems to be precisely like that of the next. As the observer continues his studies farther down stream, additional species very gradually appear, and many of the original ones likewise very gradually disappear. In any short distance these differences are so minute as to be negligible, but they are cumulative and result in an almost complete change in the flora after several hundred miles.

No ecologist would refer the alluvial forests of the upper and lower Mississippi to the same associations, yet there is no place along their whole range where one can logically mark the boundary between them. One association merges gradually into the next without any apparent transition zone. Nor is it necessary to extend our observations over such a wide area to discover this spatial variation in ecological structure. I believe no one has ever doubted that the beech-maple forest of northern Michigan constitutes a single association-type. Yet every detached area of it exhibits easily discoverable floristic peculiarities, and even adjacent square miles of a single area differ notably among themselves, not in the broader features, to be sure, but in the details of floristic composition which a simple statistical analysis brings out. In other words, the local variation in

structure of any association merges gradually into the broader geographical variation of the association-type.

This diversity in space is commonly overlooked by ecologists, most of whom of necessity limit their work to a comparatively small area, not extensive enough to indicate that the small observed floristic differences between associations may be of much significance or that this wide geographical variation is actually in operation. Yet it makes difficult the exact definition of any association-type, except as developed in a restricted locality, renders it almost impossible to select for study a typical or average example of a type, and in general introduces complexities into any attempt to classify plant associations.

What have we now as a basis for consideration in our attempts to define and classify associations? In the northeastern states, we can find many sharply marked communities, capable of fairly exact location on a map. But not all of that region can be thus divided into associations, and there are other regions where associations, if they exist at all in the ordinary sense of the word, are so vaguely defined that one does not know where their limits lie and can locate only arbitrary geographic boundaries. We know that associations vary internally from year to year, so that any definition we may make of a particular community, based on the most careful analysis of the vegetation, may be wrong next year. We know that the origin and disappearance of some are rapid, of others slow, but we do not always know whether a particular type of vegetation is really an association in itself or represents merely the slow transition stage between two others. We know that no two areas, supposed to represent the same association-type, are exactly the same, and we do not know which one to accept as typical and which to assume as showing the effects of geographical variation. We find fragmentary associations, and usually have no solid basis for deciding whether they are mere accidental intruders or embryonic stages in a developing association which may become typical after a lapse of years. We find variation of environment within the association, similar associations occupying different environments, and different associations in the same environment. It is small wonder that there is conflict and confusion in the definition and classification of plant communities. Surely our belief in the integrity of the association and the sanc-

tity of the association-concept must be severely shaken. Are we not justified in coming to the general conclusion, far removed from the prevailing opinion, that an association is not an organism, scarcely even a vegetational unit, but merely a *coincidence*?

This question has been raised on what might well be termed negative evidence. It has been shown that the extraordinary variability of the areas termed associations interferes seriously with their description, their delimitation, and their classification. Can we find some more positive evidence to substantiate the same idea? To do this, we must revert to the individualistic concept of the development of plant communities, as suggested by me in an earlier paper.<sup>3</sup>

As a basis for the presentation of the individualistic concept of the plant association, the reader may assume for illustration any plant of his acquaintance, growing in any sort of environment or location. During its life it produces one or more crops of seeds, either unaided or with the assistance of another plant in pollination. These seeds are endowed with some means of migration by which they ultimately come to rest on the ground at a distance from the parent plant. Some seeds are poorly fitted for migration and normally travel but a short distance; others are better adapted and may cover a long distance before coming to rest. All species of plants occasionally profit by accidental means of dispersal, by means of which they traverse

<sup>3</sup> I may frankly admit that my earlier ideas of the plant association were by no means similar to the concept here discussed. Ideas are subject to modification and change as additional facts accumulate and the observer's geographical experience is broadened. An inkling of the effect of migration on the plant community appeared as early as 1903 and 1904 (Bull. Illinois State Lab. Nat. Hist. 7: 189.) My field work of 1908 covered a single general type of environment over a wide area, and was responsible for still more of my present opinions (Bull. Illinois State Lab. Nat. Hist. 9: 35-42). Thus we find such statements as the following: "No two areas of vegetation are exactly similar, either in species, the relative numbers of individuals of each, or their spatial arrangement" (l. c. 37), and again: "The more widely the different areas of an association are separated, the greater are the floral discrepancies. . . . Many of these are the results of selective migration from neighboring associations, so that a variation in the general nature of the vegetation of an area affects the specific structure of each association" (l. c. 41). Still further experience led to my summary of vegetational structure in 1917 (Bull. Torrey Club 44: 463-481), and the careful quantitative study of certain associations from 1911 to 1923 produced the unexpected information that the distribution of species and individuals within a community followed the mathematical laws of probability and chance (Ecology 6: 66-74).

distances far in excess of their average journey. Sometimes these longer trips may be of such a nature that the seed is rendered incapable of germination, as in dispersal by currents of salt water, but in many cases they will remain viable. A majority of the seeds reach their final stopping-point not far from the parent, comparatively speaking, and only progressively smaller numbers of them are distributed over a wider circle. The actual number of seeds produced is generally large, or a small number may be compensated by repeated crops in successive years. The actual methods of dispersal are too well known to demand attention at this place.

For the growth of these seeds a certain environment is necessary. They will germinate between folds of paper, if given the proper conditions of light, moisture, oxygen, and heat. They will germinate in the soil if they find a favorable environment, irrespective of its geographical location or the nature of the surrounding vegetation. Herein we find the *crux* of the question. The plant individual shows no physiological response to geographical location or to surrounding vegetation *per se*, but is limited to a particular complex of environmental conditions, which may be correlated with location, or controlled, modified, or supplied by vegetation. If a viable seed migrates to a suitable environment, it germinates. If the environment remains favorable, the young plants will come to maturity, bear seeds in their turn, and serve as further centers of distribution for the species. Seeds which fall in unfavorable environments do not germinate, eventually lose their viability and their history closes.

As a result of this constant seed-migration, every plant association is regularly sowed with seeds of numerous extra-limital species, as well as with seeds of its own normal plant population. The latter will be in the majority, since most seeds fall close to the parent plant. The seeds of extra-limital species will be most numerous near the margin of the association, where they have the advantage of proximity to their parent plants. Smaller numbers of fewer species will be scattered throughout the association, the actual number depending on the distance to be covered, and the species represented depending on their means of migration, including the various accidents of dispersal. This thesis needs no argument in its support. The practical univer-

salinity of seed dispersal is known to every botanist as a matter of common experience.

An exact physiological analysis of the various species in a single association would certainly show that their optimal environments are not precisely identical, but cover a considerable range. At the same time, the available environment tends to fluctuate from year to year with the annual variations of climate and with the accumulated reactionary effects of the plant population. The average environment may be near the optimum for some species, near the physiological limit of others, and for a third group may occasionally lie completely outside the necessary requirements. In the latter case there will result a group of evanescent species, variable in number and kind, depending on the accidents of dispersal, which may occasionally be found in the association and then be missing for a period of years. This has already been suggested by the writer as a probable explanation of certain phenomena of plant life on mountains, and was also clearly demonstrated by Dodds, Ramaley, and Robbins in their studies of vegetation in Colorado. In the first and second cases, the effect of environmental variation toward or away from the optimum will be reflected in the number of individual plants and their general luxuriance. On the other hand, those species which are limited to a single type of plant association must find in that and in that only the environmental conditions necessary to their life, since they have certainly dispersed their seeds many times into other communities, or else be so far removed from other associations of similar environment that their migration thence is impossible.

Nor are plants in general, apart from these few restricted species, limited to a very narrow range of environmental demands. Probably those species which are parasitic or which require the presence of a certain soil-organism for their successful germination and growth are the most highly restricted, but for the same reason they are generally among the rarest and most localized in their range. Most plants can and do endure a considerable range in their environment.

With the continuance of this dispersal of seeds over a period of years, every plant association tends to contain every species of the vicinity which can grow in the available environment. Once a species is established, even by a single seed-bearing plant,

its further spread through the association is hastened, since it no longer needs to depend on a long or accidental migration, and this spread is continued until the species is eventually distributed throughout the area of the association. In general, it may be considered that, other things being equal, those species of wide extent through an association are those of early introduction which have had ample time to complete their spread, while those of localized or sporadic distribution are the recent arrivals which have not yet become completely established.

This individualistic standpoint therefore furnishes us with an explanation of several of the difficulties which confront us in our attempts to diagnose or classify associations. Heterogeneity in the structure of an association may be explained by the accidents of seed dispersal and by the lack of time for complete establishment. Minor differences between neighboring associations of the same general type may be due to irregularities in immigration and minor variations in environment. Geographical variation in the floristics of an association depends not alone on the geographical variation of the environment, but also on differences in the surrounding floras, which furnish the immigrants into the association. Two widely distant but essentially similar environments have different plant associations because of the completely different plant population from which immigrants may be drawn.

But it must be noted that an appreciation of these conditions still leaves us unable to recognize any one example of an association-type as the normal or typical. Every association of the same general type has come into existence and had its structure determined by the same sort of causes; each is independent of the other, except as it has derived immigrants from the other; each is fully entitled to be recognized as an association and there is no more reason for regarding one as typical than another. Neither are we given any method for the classification of associations into any broader groups.

Similar conditions obtain for the development of vegetation in a new habitat. Let us assume a dozen miniature dunes, heaped up behind fragments of driftwood on the shore of Lake Michigan. Seeds are heaped up with the sand by the same propelling power of the wind, but they are never very numerous and usually of various species. Some of them germinate, and the dozen embryonic dunes may thenceforth be held by as many different

species of plants. Originally the environment of the dunes was identical and their floristic difference is due solely to the chances of seed dispersal. As soon as the plants have developed, the environment is subject to the modifying action of the plant, and small differences between the different dunes appear. These are so slight that they are evidenced more by the size and shape of the dune than by its flora, but nevertheless they exist. Additional species gradually appear, but that is a slow process, involving not only the chance migration of the seed to the exact spot but also its covering upon arrival. It is not strange that individuals are few and that species vary from one dune to another, and it is not until much later in the history of each dune, when the ground cover has become so dense that it affects conditions of light and soil moisture, and when decaying vegetable matter is adding humus to the sand in appreciable quantities, that a true selective action of the environment becomes possible. After that time permanent differences in the vegetation may appear, but the early stages of dune communities are due to chance alone. Under such circumstances, how can an ecologist select character species or how can he define the boundaries of an association? As a matter of fact, in such a location the association, in the ordinary sense of the term, scarcely exists.

Assume again a series of artificial excavations in an agricultural region, deep enough to catch and retain water for most or all of the summer, but considerably removed from the nearest areas of natural aquatic vegetation. Annually the surrounding fields have been ineffectively planted with seeds of *Typha* and other wind-distributed hydrophytes, and in some of the new pools *Typha* seeds germinate at once. Water-loving birds bring various species to other pools. Various sorts of accidents conspire to the planting of all of them. The result is that certain pools soon have a vegetation of *Typha latifolia*, others of *Typha angustifolia*, others of *Scirpus validus*; plants of *Iris versicolor* appear in one, of *Sagittaria* in another, of *Alisma* in a third, of *Juncus effusus* in a fourth. Only the chances of seed dispersal have determined the allocation of species to different pools, but in the course of three or four years each pool has a different appearance, although the environment, aside from the reaction of the various species, is precisely the same for each. Are we dealing here with several different associations, or with a single association, or with



merely embryonic stages of some future association? Under our view, these become merely academic questions, and any answer which may be suggested is equally academic.

But it must again be emphasized that these small areas of vegetation are component parts of the vegetative mantle of the land, and as such are fully worthy of description, of discussion, and of inquiry into the causes which have produced them and into their probable future. It must be emphasized that in citing the foregoing examples, the existence of associations or of successions is not denied, and that the purpose of the two paragraphs is to point out the fact that such communities introduce many difficulties into any attempt to define or classify association-types and successional series.

A plant association therefore, using the term in its ordinarily accepted meaning, represents the result of an environmental sorting of a population, but there are other communities which have existed such a short time that a reasonably large population has not yet been available for sorting.

Let us consider next the relation of migration and environmental selection to succession. We realize that all habitats are marked by continuous environmental fluctuation, accompanied or followed by a resulting vegetational fluctuation, but, in the common usage of the term, this is hardly to be regarded as an example of succession. But if the environmental change proceeds steadily and progressively in one direction, the vegetation ultimately shows a permanent change. Old species find it increasingly difficult or impossible to reproduce, as the environment approaches and finally passes their physiological demands. Some of the migrants find establishment progressively easier, as the environment passes the limit and approaches the optimum of their requirements. These are represented by more and more individuals, until they finally become the most conspicuous element of the association, and we say that a second stage of a successional series has been reached.

It has sometimes been assumed that the various stages in a successional series follow each other in a regular and fixed sequence, but that is frequently not the case. The next vegetation will depend entirely on the nature of the immigration which takes place in the particular period when environmental change reaches the critical stage. Who can predict the future

for any one of the little ponds considered above? In one, as the bottom silts up, the chance migration of willow seeds will produce a willow thicket, in a second a thicket of *Cephalanthus* may develop, while a third, which happens to get no shrubby immigrants, may be converted into a miniature meadow of *Calamagrostis canadensis*. A glance at the diagram of observed successions in the Beach Area, Illinois, as published by Gates, will show at once how extraordinarily complicated the matter may become, and how far vegetation may fail to follow simple, pre-supposed successional series.

It is a fact, of course, that adjacent vegetation, because of its mere proximity, has the best chance in migration, and it is equally true that in many cases the tendency is for an environment, during its process of change, to approximate the conditions of adjacent areas. Such an environmental change becomes effective at the margin of an association, and we have as a result the apparent advance of one association upon another, so that their present distribution in space portrays their succession in time. The conspicuousness of this phenomenon has probably been the cause of the undue emphasis laid on the idea of successional series. But even here the individualistic nature of succession is often apparent. Commonly the vegetation of the advancing edge differs from that of the older established portion of the association in the numerical proportion of individuals of the component species due to the sorting of immigrants by an environment which has not yet reached the optimum, and, when the rate of succession is very rapid, the pioneer species are frequently limited to those of the greatest mobility. It also happens that the change in environment may become effective throughout the whole area of the association simultaneously, or may begin somewhere near the center. In such cases the pioneers of the succeeding association are dependent on their high mobility or on accidental dispersal, as well as environmental selection.

It is well known that the duration of the different stages in succession varies greatly. Some are superseded in a very short time, others persist for long or even indefinite periods. This again introduces difficulties into any scheme for defining and classifying associations.

A forest of beech and maple in northern Michigan is lumbered, and as a result of exposure to light and wind most of the usual

herbaceous species also die. Brush fires sweep over the clearing and aid in the destruction of the original vegetation. Very soon the area grows up to a tangle of other herbaceous and shrubby species, notably *Epilobium angustifolium*, *Rubus strigosus*, and *Sambucus racemosa*. This persists but a few years before it is overtopped by saplings of the original hardwoods which eventually restore the forest. Is this early stage of fire-weeds and shrubs a distinct association or merely an embryonic phase of the forest? Since it has such a short duration, it is frequently regarded as the latter, but since it is caused by an entirely different type of environmental sorting and lacks most of the characteristic species of the forest, it might as well be called distinct. If it lasted for a long period of years it would certainly be called an association, and if all the forest near enough to provide seeds for immigration were lumbered, that might be the case. Again we are confronted with a purely arbitrary decision as to the associational identity of the vegetation.

Similarly, in the broad transition zone between the oak-covered mountains and the grass-covered foothills in the Coast Range of California, we are forced to deal arbitrarily in any matter of classification. Shall we call such a zone a mere transition, describe the forests above and the grasslands below and neglect the transition as a mere mixture? Or shall we regard it as a successional or time transition, evidencing the advance of the grasslands up the mountain or of the oaks down toward the foothills? If we choose the latter, we must decide whether the future trend of rainfall is to increase, thereby bringing the oaks to lower elevations, or to decrease, thereby encouraging the grasslands to grow at higher altitudes. If we adopt the former alternative, we either neglect or do a scientific injustice to a great strip of vegetation, in which numerous species are "associated" just as surely as in any recognized plant association.

The sole conclusion we can draw from all the foregoing considerations is that the vegetation of an area is merely the resultant of two factors, the fluctuating and fortuitous immigration of plants and an equally fluctuating and variable environment. As a result, there is no inherent reason why any two areas of the earth's surface should bear precisely the same vegetation, nor any reason for adhering to our old ideas of the definiteness and distinctness of plant associations. As a matter of fact, no

two areas of the earth's surface do bear precisely the same vegetation, except as a matter of chance, and that chance may be broken in another year by a continuance of the same variable migration and fluctuating environment which produced it. Again, experience has shown that it is impossible for ecologists to agree on the scope of the plant association or on the method of classifying plant communities. Furthermore, it seems that the vegetation of a region is not capable of complete segregation into definite communities, but that there is a considerable development of vegetational mixtures.

Why then should there be any representation at all of these characteristic areas of relatively similar vegetation which are generally recognized by plant ecologists under the name of associations, the existence of which is indisputable as shown by our field studies in many parts of the world, and whose frequent repetition in similar areas of the same general region has led us to attempt their classification into vegetational groups of superior rank?

It has been shown that vegetation is the resultant of migration and environmental selection. In any general region there is a large flora and it has furnished migrating seeds for all parts of the region alike. Every environment has therefore had, in general, similar material of species for the sorting process. Environments are determined principally by climate and soil, and are altered by climatic changes, physiographic processes, and reaction of the plant population. Essentially the same environments are repeated in the same region, their selective action upon the plant immigrants leads to an essentially similar flora in each, and a similar flora produces similar reactions. These conditions produce the well known phenomena of plant associations of recognizable extent and their repetition with great fidelity in many areas of the same region, but they also produce the variable vegetation of our sand dunes and small pools, the fragmentary associations of areas of small size, and the broad transition zones where different types of vegetation are mixed. Climatic changes are always slow, physiographic processes frequently reach stages where further change is greatly retarded, and the accumulated effects of plant reaction often reach a condition beyond which they have relatively little effect on plant life. All of these conspire to give to certain areas a comparatively uniform en-

vironment for a considerable period of time, during which continued migration of plants leads to a smoothing out of original vegetational differences and to the establishment of a relatively uniform and static vegetational structure. But other physiographic processes are rapid and soon develop an entirely different environment, and some plant reactions are rapid in their operation and profound in their effects. These lead to the short duration of some plant communities, to the development, through the prevention of complete migration by lack of sufficient time, of associations of few species and of different species in the same environment, and to mixtures of vegetation which seem to baffle all attempts to resolve them into distinct associations.

Under the usual concept, the plant association is an area of vegetation in which spatial extent, describable structure, and distinctness from other areas are the essential features. Under extensions of this concept it has been regarded as a unit of vegetation, signifying or implying that vegetation in general is composed of a multiplicity of such units, as an individual representation of a general group, bearing a general similarity to the relation of an individual to a species, or even as an organism, which is merely a more striking manner of expressing its unit nature and uniformity of structure. In every case spatial extent is an indispensable part of the definition. Under the individualistic concept, the fundamental idea is neither extent, unit character, permanence, nor definiteness of structure. It is rather the visible expression, through the juxtaposition of individuals, of the same or different species and either with or without mutual influence, of the result of causes in continuous operation. These primary causes, migration and environmental selection, operate independently on each area, no matter how small, and have no relation to the process on any other area. Nor are they related to the vegetation of any other area, except as the latter may serve as a source of migrants or control the environment of the former. The effect of these primary causes is therefore not to produce large areas of similar vegetation, but to determine the plant life on every minimum area. The recurrence of a similar juxtaposition over tracts of measurable extent, producing an association in the ordinary use of the term, is due to a similarity in the contributing causes over the whole area involved.

Where one or both of the primary causes changes abruptly, sharply delimited areas of vegetation ensue. Since such a condition is of common occurrence, the distinctness of associations is in many regions obvious, and has led first to the recognition of communities and later to their common acceptance as vegetational units. Where the variation of the causes is gradual, the apparent distinctness of associations is lost. The continuation in time of these primary causes unchanged produces associational stability, and the alteration of either or both leads to succession. If the nature and sequence of these changes are identical for all the associations of one general type (although they need not be synchronous), similar successions ensue, producing successional series. Climax vegetation represents a stage at which effective changes have ceased, although their resumption at any future time may again initiate a new series of successions.

In conclusion, it may be said that every species of plant is a law unto itself, the distribution of which in space depends upon its individual peculiarities of migration and environmental requirements. Its disseminules migrate everywhere, and grow wherever they find favorable conditions. The species disappears from areas where the environment is no longer endurable. It grows in company with any other species of similar environmental requirements, irrespective of their normal associational affiliations. The behavior of the plant offers in itself no reason at all for the segregation of definite communities. Plant associations, the most conspicuous illustration of the space relation of plants, depend solely on the coincidence of environmental selection and migration over an area of recognizable extent and usually for a time of considerable duration. A rigid definition of the scope or extent of the association is impossible, and a logical classification of associations into larger groups, or into successional series, has not yet been achieved.

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## Notes on *Tilia*

W. W. ASHE

### PUBESCENT MIDSUMMER SHOOTS

In the characterization of the tree of southwestern Louisiana described under the name *Tilia Cocksii* Dr. Sargent<sup>1</sup> calls attention to the unique feature whereby a glabrous or nearly glabrous spring shoot is terminated by pubescent or tomentose midsummer growth. This manner of development is not confined to this species but is apparently common to at least four other lindens and probably should be regarded as a group character. The tomentose midsummer growth is followed by a glabrous shoot the following spring. The result is a branchlet having alternate sections glabrous and tomentose or having a tomentose internode. It is desirable that collections of summer shoots be made of other southern lindens. It might seem to be designed as a means of protecting the tender midsummer growth which develops during a period of high temperature from injury by the heat. The other species so far noted having this character are *Tilia porracea* Ashe,<sup>2</sup> *T. leucocarpa* Ashe,<sup>3</sup> *T. floridana* Small,<sup>4</sup> and *T. crenato-serrata* Sargent.<sup>5</sup> This is a character which divides the glabrous species of this genus, or the species with glabrous twigs, in the eastern United States into two sections.

Since the preceding paragraph was written, it has been possible to examine summer shoots of *Tilia venulosa* Sargent (Bot. Gaz. 66: 426. 1918) on a tree which the writer has growing. The shoot is pubescent with long ascending straight hairs mixed with shorter fascicled hairs. Thus five species having glabrous spring shoots have pubescent summer shoots. It should be recorded here that summer shoots of *Tilia glabra* Vent. (Anales Hist. Nat. 2: 62. 1800) from trees growing in Washington, D. C., are glabrous. *T. alabamensis* Ashe (Bull. Charleston Museum 14: 31. 1918) and *T. australis* Small also have glabrous summer shoots.

<sup>1</sup> Bot. Gaz. 66: 437. 1918.

<sup>2</sup> Charleston Museum Quart. 1: 31. 1925.

<sup>3</sup> Bull. Charleston Museum 14: 32. 1918.

<sup>4</sup> Fl. Southeastern United States 761. 1903.

<sup>5</sup> Bot. Gaz. 66: 430. 1918.

## TILIA MICHAUXII Nuttall

F. A. Michaux,<sup>6</sup> in volume three of his work on the trees of North America, described three species of *Tilia* in the following sequence: *Tilia americana*, *T. alba*, and *T. pubescens*. Of these the first and last were included in the Flora Boreali-Americana of André Michaux,<sup>7</sup> his father, the former under the name *T. canadensis* and the latter as *T. laxiflora*. *T. alba* was proposed as a new species, its description being supplemented by an excellent plate. In referring to the distribution and habitat of the proposed species, it is stated that "it is common in Pennsylvania, Maryland, and Virginia and in the states west of the Allegheny Mountains. This lime does not grow, like the preceding [i.e. *T. americana*], on uplands, nor among other trees in the forests; it is seldom seen elsewhere than on the banks of rivers" (translated). The distribution which is given and the habitat assigned seem to have been important in determining the conception of the tree which is described. The common linden throughout this region in the habitat given is the species which recent authors refer to *T. Michauxii* Nuttall. Its characters are well known: leaves of medium size, 7 to 10 cm. long, ovate, prevalingly cordate at base, short petiole, 3 to 4 cm. long, medium green above, pale grayish-green beneath with a close scattered stellate pubescence especially on leaves from the lower branches, or sometimes the leaves on upper part of tree white beneath with an extremely short, close, but never dense tomentum, flowers 6 to 7 mm. long, bract narrow, 1 to 2 cm. wide but often elongated, 10 to 12 cm. long.

Now *Tilia Michauxii* Nuttall<sup>8</sup> was based on *T. alba* Michaux fils, which name was invalid because of the earlier *T. alba* L. The description of *T. alba* Michx. f. (translated and only essential characters mentioned) is quite different from that just given: "The leaves are very large, oval or roundish, heart-shaped or very obliquely truncate at base, upper surface dark green, lower white with little tufts of reddish hairs in the axils of the principal veins. The flowers as well as the floral bracts are larger than those of any other linden which I know. The petals also are whiter and larger." A comparison with *T. americana*, the de-

<sup>6</sup> Hist. Arb. Am. 3: 315. pl. 2. 1813.

<sup>7</sup> Fl. Bor. Am. 1: 306. 1803.

<sup>8</sup> N. A. Sylva 1: 92. 1842.



scription of which immediately precedes that of *T. alba* (and which might possibly include *T. neglecta* Spach), would indicate that the leaves, flowers, floral bracts, and petals were larger than those of that species. The flowers of *T. americana* and of *T. neglecta* are from 8 to 10 mm. long. Those of *T. alba* consequently must be larger. As figured they are about one-fourth larger, while the petioles are figured as 5 to 6 cm. long.

The only species in this general region to which the description of *T. alba* Michx. f. can apply is the Appalachian species which long passed under the name of *T. heterophylla*. It has the very large leaves described and the long petioles figured by Michaux for his *T. alba*, with the same shapes, cordate in the roundish form, very oblique in the oval, dark green above, always snowy white beneath with a close persistent tomentum and with minute reddish axillary tufts, the largest floral bracts of any eastern American species (often 18 cm. long and more than 3 cm. wide), the largest flowers (10 to 12 mm. long) with the whitest petals.

The description of *T. alba* Michx. f. clearly does not refer to *T. Michauxii* as currently interpreted and does not even include this *T. Michauxii*. It is evidently drawn exclusively from the large-leaved, large-flowered, large-bracted Appalachian species. André Michaux, the father of F. A. Michaux, between 1787 and 1796 made extensive collections of American plants and seeds which were sent to France. Many of these collections were made in the Appalachian region and in his Journal he particularly mentions collecting near the site where now is located the town of Highlands, North Carolina, a section where the large-flowered linden is common. Ventenat undoubtedly employed material from such cultivated plants introduced through Michaux and Fraser in preparing the plate which accompanies the description of his *T. heterophylla*.<sup>9</sup> Michaux fils probably drew upon such a living tree introduced by his father for this description and plate of his *T. alba*; or on the two trips he made to the United States in 1802 and 1806 preparatory to writing the "History," a tree of the large-flowered species may have been the one selected for comparison and description.

*Tilia Michauxii* is apparently distributed from near Highlands, Macon County, North Carolina, to Nashville, Tennessee,

<sup>9</sup> Mém. Inst. Sci. Paris 4<sup>e</sup>: 16. pl. 5. 1802.

and northward to Breathett County, Kentucky (W. W. A.), and Upshur County, West Virginia.

Its synonymy seems to be:

*T. alba* Michx. f. Hist. Arb. Am. 3: 315. *pl.* 2. (not Linnaeus) 1813.

*T. monticola* Sargent, Bot. Gaz. 66: 508. 1918.

*T. heterophylla*, at least in part, of many American authors.

#### TYPE OF *TILIA HETEROPHYLLA* VENTENAT

In reference to the type of *T. heterophylla* Vent., which it is believed Dr. Sargent<sup>10</sup> has established, it is significant that A. Michaux in his Journal refers to *Tilia* only in one place east of the Allegheny Mountains and south of Pennsylvania. This was under date of May 28, 1787, in South Carolina within 3 miles of Augusta, Ga. He leaves a blank after the single word "*Tilia*" in the Journal as if intending to fill in later with a description. Dr. Sargent cites near Augusta, Georgia, as a location for material which he regards as typical. This is possibly the source of the trees upon which Ventenat based his description. Material from several trees along Chauga River, 15 miles from Walhalla, South Carolina, clearly the same as the Walhalla trees (which Dr. Sargent regards as typical) conforms in the shape of the leaves to Ventenat's plate even more closely than do the Walhalla trees. None of the leaves of these trees are brownish tomentose beneath. This character, however, is not constant.

***Tilia heterophylla tenera*** comb. nov.—*T. tenera* Ashe, Bull. Charleston Museum 13: 27. 1917.—The next available name for the tree which has been called *T. heterophylla Michauxii* (Sargent, *op. cit.*, 506) seems to be *T. tenera*, which was based upon material from near the Deep River in Moore County, North Carolina.

***Tilia lata*** sp. nov. A tree up to 20 m. high and 5 dm. in diameter or occasionally almost shrubby and bearing flowers and fruiting at a height of not more than 2.2 m. Twigs rather slender, 2 to 3 mm. thick, when mature dull chestnut brown, as are the obtuse winter buds, both covered with a usually thin grayish-brown, scurfy, fascicled pubescence, mostly persistent on the buds, sometimes deciduous on the twigs by midsummer. Leaves ovate or broadly ovate, ample, the blades 11 to 16 cm. long, 10 to 16 cm. wide, abruptly acuminate at apex, truncate or ob-

<sup>10</sup> Bot. Gaz. 66: 504. 1918.

liquely cordate at base or sometimes nearly all leaves cordate, but lower invariably more cordate and symmetrical than upper, coarsely dentate with rounded or obtuse apiculate teeth, on unfolding a deep purple-bronze and pubescent above with fascicled hairs, when mature thick, dark green and glabrous above, white or bright silvery gray below with a rather close fascicled pubescence, the midrib covered with short fascicled scurfy pubescence, often mixed with long straight appressed hairs, leaves at the ends of twigs often tawny or brownish beneath; petiole short, 2 to 5 cm. long, slender, at first covered with scurfy grayish pubescence. Flowers 7 to 8 mm. long, appearing about the first week in June, in rather compact 10 to 20-flowered pubescent corymbs on slender tomentose pedicels; sepals ovate, acute, flesh colored, finely pubescent without, densely silky tomentose within or glabrous in the center; petals broadly lanceolate, contracted above the middle, the apex broad and rounded; staminodia oblong or elliptic, very broad and obtuse at apex, about two-thirds as long as petals; peduncle slender, pubescent, becoming free near the middle of the bract and sometimes extending beyond the bract, the free portion often soon glabrous; bract oblong or oblong-obovate, rounded at the broad apex, often narrow at base, the blade 8 to 12 cm. long, 2.5 to 4 cm. broad, thin, above glabrous or soon glabrous and often glaucescent, below yellowish and covered with very short fascicled pubescence, usually on a slender stalk which may be 4 cm. long, sometimes subsessile.

Type collected by the writer, May 1923, from near Sipsey Fork Bridge on the road from Addison to Houston, Alabama. Leaves on vigorous shoots sometimes 3-lobed or notched. This species is very common on the Brushy Fork of Sipsey River in Lawrence and Winston counties, Alabama, and is generally spread over the adjoining mountainous counties in the Sand Mountain region, growing particularly along streams, but occasionally on rocky hillsides well up the slopes. It is associated with *Tilia australis* Small, and blooms at the same time as that species. The proposed species differs from *T. heterophylla* Ventenat, in its larger flowers, very broad petals and staminodia, in its much shorter pubescence, in the large thin bracts, glabrous or glabrate above, and merely pubescent beneath, and in its earlier time of blooming.

*Tilia eburnea lasioclada* comb. nov.—*T. lasioclada* Sargent, Bot. Gaz. 66: 502. 1918.—This differs from the type in having the twigs more or less densely clothed with straight, simple hairs. Typical *T. eburnea* Ashe has rather stout, glabrous

twigs and medium sized leaves obliquely cordate or truncate at base, silvery pubescent beneath with loose fascicled hairs or becoming glabrate with age. The floral bract both of the type and of the variety is soft pubescent beneath with long, simple, straight, white hairs. *T. cburnea* and its variety *lasioclada* bear a superficial resemblance to *T. heterophylla tenera* from which, however, as well as from all forms of *T. heterophylla* and from all other species of the eastern United States they are separated by the simple, straight hairs on the lower surface of the bract. Pubescence when present on the bract of other species is of fascicled hairs.

**TILIA NEGLECTA** Spach. This species can be separated from all other known species of eastern North America by the straight simple firmly attached pubescence on the lower surface of the leaves. Notwithstanding that this pubescence is soft and velvety to the touch, the leaves are green or slightly grayish beneath. The pubescence on the peduncle and on the bract when present is of fascicled hairs, however.

**KEY TO SPECIES OF TILIA IN EASTERN UNITED STATES HAVING  
LOWER SURFACE OF LEAVES TOMENTOSE OR QUITE PUBESCENT  
AT FLOWERING TIME.**

- A. Pubescence or tomentum on lower surface of bract, if present, fascicled, close, soon becoming short; pubescence or tomentum on lower surface of leaves of fascicled hairs.
  - a. Midrib glabrous or nearly so at flowering time.
    - b. Flowers large, 10 to 12 mm. long. Tomentum on lower surface of leaves always white, dense and close but not velvety; twigs stout. . . . . *T. Michauxii*
    - b. Flowers smaller, 4 to 8 mm. long, twigs slender.
      - Leaves pubescent, terminal on shoots about twice as long as broad, never cordate or truncate at base, summer shoots tomentose, spring shoots glabrate. . . . . *T. parvacea*
      - Terminal leaves on shoots relatively broader.
        - Bract at flowering time pubescent beneath, tomentum on lower surface of leaves soft and velvety, leaves at tips of twigs often brownish beneath.
          - Twigs glabrous. . . . . *T. heterophylla*
          - Twigs pubescent. . . . . *T. heterophylla nivea*
  - Bract at flowering time glabrate or merely pubescent beneath. Leaves on upper branches closely white-

- tomentose beneath, lower merely pubescent, greenish or grayish. . . . *T. heterophylla tenera*
- Bract oblong, rounded at both ends, at flowering time velvety tomentose beneath, pubescent above, leaves closely white-tomentose beneath. . . . *T. apposita*
- a.* Midrib tomentose or thickly pubescent below with stellate hairs, leaves very pubescent above while unfolding.
- Leaves sharply dentate, often cordate, tomentose beneath, tomentum white or brown, but not a dusty brown, twigs tomentose, bract glabrous above. . . . *T. lata*
- Leaves sharply dentate, very oblique, pubescence beneath dusty brown, bract glabrous above. . . . *T. caroliniana*
- Leaves round-dentate, mostly cordate, covered beneath with dusty brown tomentum, bract pubescent above. . . . *T. georgiana*
- A.* Pubescence on lower surface of bract of long soft persistent white straight hairs; tomentum on lower surface of leaf loose and easily detached, fascicled.
- Twigs glabrous at flowering time. . . . *T. eburnea*
- Twigs pubescent with long straight hairs, leaves with a thick pubescence beneath. . . . *T. eburnea lasioclada*
- A.* Pubescence on lower surface of bract and on inflorescence, if present, fascicled; pubescence on lower surface of leaves of short, straight, firmly attached hairs. . . . *T. neglecta*

Since incorrect citations of some of the species in the key have recently been published, it will perhaps be a convenience to have the following verified references:

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- T. APPPOSITA* Ashe, *Bull. Charleston Museum* 13: 27. 1917.
- T. LATA* Ashe (see above).
- T. CAROLINIANA* Miller, *Gard. Dict. Ed.* 8. 1768. Sargent, *Bot. Gaz.* 66: 496. 1918.
- T. GEORGIANA* Sargent, *Bot. Gaz.* 66: 510. 1918.
- T. EBURNEA* Ashe, *Bot. Gaz.* 33: 231. 1902.
- T. EBURNEA LASIOCLADA* (Sargent) Ashe (see above).
- T. NEGLECTA* Spach, *Ann. Sci. Nat. Bot.* II. 2: 340. *pl.* 15. 1834. Sargent, *Bot. Gaz.* 66: 494. 1918.



## New plants from Colorado

GEORGE E. OSTERHOUT

**Atriplex virgata** sp. nov. Annual, the stem terete and smooth, 3-6 dm. high, numerous branches above the middle of the stem 2-4 cm. long, though much longer on some of the plants; stem and leaves only moderately scurfy; the leaves crowded on the short branches, generally alternate, mostly ovate, 1-3 cm. long, 5-12 mm. wide, many smaller, cuneate at base, obtuse at the apex, entire or with a few small teeth, 3-nerved, sessile or the peduncles very short; fruiting bracts 1-4 in the axils of the leaves, and for the most part concealed by the leaves, 5 mm. wide at the base, the same in length, toothed on the sides at the base, though somewhat variable in this respect, the faces smooth, the pedicels very short; the seeds about 2 mm. in diameter, the radicle inferior.

Type collected by the writer, no. 6520, 5 October 1925, along the ravine south of Horsetooth Mountain, Larimer County, Colorado, growing in the shade of wild plum trees. No staminate flowers were evident at the time of collecting, but it has the appearance of being monoecious. The fruiting bracts are much like those of *A. rosea* L., but without appendages on the faces.

**Sophia glandifera** sp. nov. About 3 dm. high, light green, branched from the base with strongly ascending branches which equal, or almost equal, the main stem, the stem and the leaves dotted with small, very short-stalked glands; the lower leaves bipinnate, oblanceolate in outline, 4 cm. long including the petiole, the upper leaves sessile, pinnate with narrow segments; the pedicels ascending, 1 cm. long; the pods 8-10 mm. long, clavate, glabrous, 1-1.5 mm. thick; the seeds biserial; the flowers light yellow, the sepals 2 mm. long, the petals 2.5 mm. long, spatulate; the style sessile.

Type collected by the writer, no. 5226, 27 May 1915, at Hayden, Routt County, Colorado—just south of the town. The glandular character of the plant distinguishes it from other species of *Sophia*.

**Miltitzia pinnatifida** sp. nov. Annual, 6-15 cm. high, divided near the base into 2-4 branches which attain to nearly the same height, the whole plant hirsute; the leaves rather few,

oblanceolate in outline, 2-3 cm. long, divided half way to the midrib or almost to the midrib, the divisions oblong, obtuse, 3-4 mm. long; the inflorescence taking nearly the upper half of the plant with scorpioid racemes of numerous flowers and fruiting capsules, the calyx divided to the base, the lobes linear, obtuse, 2.5 mm. long in flower, becoming 6-8 mm. long in fruit, hirsute, two of the lobes a little larger than the others; the corolla the length of the calyx lobes, white or tinged with blue, the lobes small and rounded; the style 1 mm. long, divided near the top; the capsule 4 mm. long, 2-celled, splitting from the top into 2 valves; the seeds 15-20 in each capsule, 1 mm. long with 6-7 sharp cross ridges.

Type collected by the writer, *no.* 6393, 18 June 1925, west of Craig, Moffat County, Colorado—in sandy soil.

*PTILORIA BIGELOVII* (A. Gray) Woot. & Standl. *Contr. U. S. Nat. Herb.* 16: 176. 1913. What I take to be this plant was collected in Moffat County, northwestern Colorado, a few miles east of the Utah line, June 20, 1925. So far as I know it has not been reported north of New Mexico.



# INDEX TO AMERICAN BOTANICAL LITERATURE

1924-1925

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Reviews, and papers that relate exclusively to forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included, and no attempt is made to index the literature of bacteriology. An occasional exception is made in favor of some paper appearing in an American periodical which is devoted wholly to botany. Reprints are not mentioned unless they differ from the original in some important particular. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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*Terminalia* in the lower Eocene of southeastern North America

EDWARD W. BERRY

(WITH FIGURES 1-6)

During the field studies of E. W. Hilgard, which culminated in his classic volume on the Geology and Agriculture of the State of Mississippi, published in 1860, he encountered fossil plants at several localities. These he collected and submitted to Leo Lesquereux, the nestor of American paleobotanists. The latter's botanical determinations of these fossil plants are listed in connection with Hilgard's account of the stratigraphy of Mississippi.

Among the lists of fossil plants occurs a species of *Terminalia* (p. 113), obviously based upon pictures of leaves which European students of Tertiary floras had referred to that genus, and not upon comparisons with the leaves of existing species of *Terminalia*. The leaf from Mississippi so-named by Lesquereux, of large size and characteristic in form and venation, has since been found to have a considerable geologic and geographic range.

When Lesquereux came to publish an illustrated systematic account of Hilgard's collections, which he did in 1869, he had changed his opinion of the relationship of this particular species and transferred it to the genus *Magnolia*,<sup>1</sup> a member of a different and quite unrelated family, and thereafter he consistently referred all large and simple fossil leaves to the genus *Magnolia*, in which practise he has been followed by Newberry, Hollick, and Knowlton.

When I was preparing the account of the Wilcox flora which was published in 1916,<sup>2</sup> I compared these and other related

<sup>1</sup> Lesquereux, Leo. Trans. Am. Philos. Soc. 13: 421. 1869.

<sup>2</sup> Berry, Edward W. The lower Eocene floras of southeastern North America. U. S. Geol. Survey Prof. Paper 91: 1-481. pl. 1-117. 1916.

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leaves with those of recent species of *Terminalia* and *Magnolia*, and reached the conclusion that, in all of their essential features, they resembled the former and differed from the latter genus. This conclusion, necessarily not beyond doubt when based upon the foliage alone, was corroborated by finding in association with the leaves described as *Terminalia Hilgardiana* (Lesquereux) Berry, nut-like fruits which were very similar to those of existing *Terminalias* that have dry wingless fruits like those of *Terminalia Catappa* L., and these were described under the name *Terminalia Wilcoxiana* Berry. These fruits and the leaves of *T. Hilgardiana* have been utilized in making a restoration of a fruiting branch, which will be published eventually in an elaborate account of the Wilcox flora now in course of preparation.

A somewhat less important argument for regarding these leaves as those of *Terminalia* in preference to *Magnolia* is furnished by the fact that nowhere among the tons of Wilcox plant material that has passed through my hands have I found any traces of seeds or fruits which could be referred to *Magnolia*. It is true that certain leaves from the Wilcox have been recorded as so-called species of *Magnolia*, but this seeming inconsistency resulted from identifications of forms in the Wilcox which had been described by others from outside the Atlantic Coastal Plain, and which were of considerable chronologic interest, and about whose botanical relationship I was at a loss.

Still another feature of some weight is furnished by the botanical facies of the Wilcox flora as a whole, and particularly the facies of the richest and best represented horizon in the Wilcox, namely toward the top of the Holly Springs sand. Here the dominant ecological group is a plant association, which might properly be termed beach jungle except for the fact that this term has been used by students of the recent flora almost entirely for such a grouping on tropical strands, and the Wilcox strand was not tropical. In this environment, which has been determined with considerable precision by inductive and not subjective logic, *Terminalia* would be much more likely to be represented than would *Magnolia*.

There is therefore every line of evidence leading away from *Magnolia* and toward recognizing *Terminalia*, and this identification has received additional confirmation during the past year by the discovery, at four different localities near the



top of the Holly Springs sand in western Kentucky and Tennessee, of a second and entirely distinctive type of *Terminalia* fruit. It is the purpose of the present note to name and describe this fruit, and to present a restoration based upon it and combined with the associated foliage known as *Terminalia Lesleyana* (Lesquereux) Berry. The new species based upon fruits may be described as follows:

***Terminalia vera* Berry, n. sp. (FIGS. 1-5)**

Fruits elongate, bi-alate, elliptical in outline, pedunculate; consisting of an axial, thickened seed cavity, fusiform in shape, ligneous in appearance as preserved as a carbonaceous impression. This seed cavity extends from near the base, almost to the tip of the fruit, and occupies from  $\frac{1}{3}$  to  $\frac{1}{2}$  of its transverse diameter. The wings are scarious, very finely veined, truncated or rounded proximad, and more or less emarginate distad, with entire but slightly irregular margins. The venation is thin and largely immersed in the wing substance, which is of considerable consistency; it is reticulate and approximately of one caliber throughout; over the seed cavity it is prevaillingly of longitudinally elongated meshes, the long axis of the meshes curving outward toward the margins of the seed cavity where they pass to the wings. On the lower part of the wings, and correspondingly near the base of the seed cavity, the long axis of the meshes is transverse, and these become more ascending on the wings as the distance above their base increases.

These fruits vary considerably in size and in the degree of elongation of their elliptical outline. The peduncle is short, stout, generally curved, and about 4 mm. in length; it is usually broken off before fossilization, but is preserved in some specimens. The fruit itself varies from 1.7 to 3.5 cm. in length, and from 0.9 to 1.5 cm. in maximum width, which is midway between the apex and the base.

These fruits are readily distinguishable from the somewhat similar fruits found in the Wilcox deposits, which have been referred to the genera *Ptelea* and *Dodonaea*; and to the botanically unassigned species, *Carpolithus prangosoides* Berry, so that it is unnecessary to enumerate the differences in the present connection. They agree in all of their features with the fruits of the existing species of *Terminalia* which have bi-alate fruits, and there is not the slightest doubt but they represent a lower Eocene species of that genus. They have been discovered at the following localities in three adjacent counties: Grable pit and

Puryear, Henry County, Tennessee; Thompson & Barksdale prospect, 2 miles south of McKenzie, Carroll County, Tennessee; and 100 yards East of the Bell City Pottery pit, Calloway County, Kentucky.

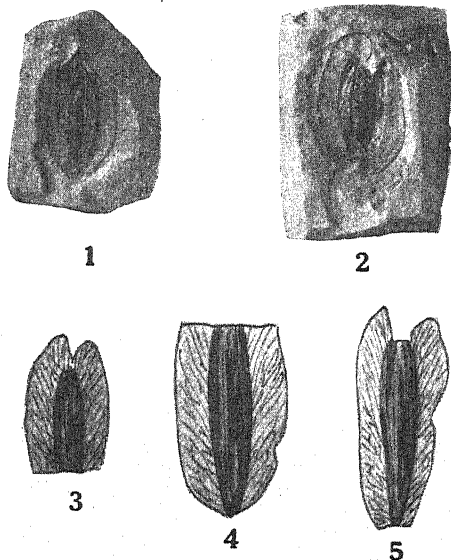
I have made comparisons with as many winged fruits of *Terminalia* as I could obtain access to, although I have seen only about ten per cent of all of the existing species. All of the American bi-alate forms that I have seen have the wings transversely extended, so that the fruits are much wider than they are high. On the other hand existing forms with the proportions of the fossil are to be found in both African species, as for example *Terminalia Brownei* Fresen. of Abyssinia; and Asiatic species, as for example, *Terminalia Darlingii* Merrill of the Philippine Islands. In fact the last is extremely like the fossil in every way. Whether the greater resemblance of this lower Eocene American species to existing African and Asiatic forms has any significance, or whether there are existing American species with fruits of this character I cannot say—I have not seen any.

The genus *Terminalia* comprises over 100 existing species, found in all tropical countries, and usually segregated into four sections according as the fruit is fleshy, nut-like and ligneous, or variously winged. The present fossil form obviously belongs in the section Diptera, which is represented in modern floras in South America, Asia, and Africa.

The family to which *Terminalia* belongs is usually termed the Combretaceae, although it is often called the Terminaliaceae. It includes about 16 genera and nearly 300 existing species of shrubs, trees, and tropical vines; with simple, entire, coriaceous, persistent, exstipulate, alternate or opposite leaves, which are often of large size. The inflorescence is racemose or capitate; and the flowers are regular, perfect or polygamous, often apetalous. The stamens are two or three times as numerous as the petals, and the one-celled ovary develops into a drupaceous or berry-like, often ligneous or winged, indehiscent fruit, which contains a single seed without endosperm, and is crowned with the accrescent calyx in many species.

The existing species are all tropical or sub-tropical, ranging from 34° north to 35° south latitude; and a relatively large number are littoral or strand types. The various continental areas contain the following number of peculiar species: America

75, Africa 85, Madagascar 36, Asia 57, Australia 23. About ten or a dozen species are found in more than one of these areas, and there is a remarkable number of identical or closely related species in tropical west Africa and tropical America, the genera *Cacoucia*, *Conocarpus*, and *Laguncularia* having identical species in both regions.



FIGS. 1-5. Fruits of *TERMINALIA VERA* Berry, n. sp. 1, from Grable pit, Tenn. 2, from 100 yards east of Bell City Pottery pit, Kentucky. 3-5, from Puryear, Tennessee.

The geological history of the family is exceedingly incomplete and will doubtless remain so until the geology of the equatorial regions is more thoroughly explored. At least the genera *Terminalia*, *Conocarpus*, *Combretum*, and *Laguncularia* have been found fossil, and all of these are represented in the Wilcox flora, where both leaves and fruit of *Terminalia*, and both leaves and flowers of *Combretum* have been discovered.

No genus of the family has been identified with certainty from rocks earlier than the Tertiary, although leaves referred to *Conocarpites* have been described from the Upper Cretaceous (Tuscaloosa formation) of Alabama, others referred to *Terminaliphyllum* from the Upper Cretaceous (Perucur beds) of

Bohemia, and still others have been referred to *Combretiphyllum* from the Upper Cretaceous of western Africa (Kamerun).

In addition to the two kinds of leaves and two kinds of fruits of *Terminalia* found in the Wilcox Eocene, the genus is represented by leaves of two species in the middle Eocene (Claiborne group), and one of the latter continues into the upper Eocene (Jackson group) in this same region. The foregoing represent the earliest authentic occurrences of the genus, although it is represented by undescribed material from the middle Eocene of Italy. During succeeding Oligocene time five or six species of *Terminalia* are recorded from the northern shores of the Mediterranean in southeastern France, the Tyrol, Italy, Styria, Carniola, and Greece. About seven species have been described from the Miocene in Switzerland, Germany, Italy, Croatia, Bohemia and Hungary in Europe; and from the Island of Trinidad, and southern Chile in South America. Pliocene species have been recorded from Spain and Italy in Europe; and the characteristic winged fruits of two species are present in the Pliocene of Bolivia (Potosi and Corocoro).

The accompanying restoration, one third natural size, is based upon a careful study of the habits of various existing species (FIG. 6). The leaves are those of *Terminalia Lesleyana* (Lesquereux) Berry, which is found in the pre-Wilcox Eocene of Texas, the Raton formation of the southern Rocky Mountain Front range, and from the bottom to the top of the Wilcox group. With these leaves I have associated the bi-alate fruits described in the preceding paragraphs. The fruits and leaves have not been found in association at the same outcrop, but both occur at identical horizons in the Wilcox.

The only uncertainty about the restoration is the use of the leaves of the *Terminalia Lesleyana* type rather than those of the *Terminalia Hilgardiana* type. The latter are equally widespread in the Wilcox, but since they are associated with the nut-like fruits of *Terminalia Wilcoxiana*, I have assumed that the last two probably represented a single botanical species. In any event the two types of leaves are not very dissimilar, so that I believe that the restoration presents an essentially correct idea of the appearance of a lower Eocene form of this interesting genus, one that is far more objective than would be the mere portrayal of single detached leaves and fruits.



FIG. 6. Restoration of a lower Eocene species of *TERMINALIA* from the Mississippi embayment region,  $\frac{1}{3}$  natural size.



## Botanical problems of American tropical agriculture<sup>1</sup>

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In the field of the plant sciences there are nowhere so many unsolved problems as in the Tropics. There the frontier offers to the pioneer investigator a chance to open new roads to knowledge. There we find outstanding opportunities for service by science in the interest of progress in two hemispheres, that have become closely interdependent.

The people of tropical countries derive their principal livelihood from agriculture, and the products of their fields and forests form the greater part of their export trade. Their future development will continue along agricultural lines, and their expanding production of raw materials will be sent north to be worked up in our factories or to feed an increasing urban population. The United States, on the other hand, has become an industrial nation, dependent on the outside world for many essentials, and will need more and more to buy plant products from the Tropics, in exchange for manufactures.\*

Our tropical importations are, in fact, peculiarly indispensable threads in the interwoven fabric of our modern civilization. Consider rubber and other gums and resins; coffee, tea, and chocolate; sugar; oils; fruits and nuts; the fibers, sisal, jute, abaca; dyes; spices; and woods.

Our annual purchases of these important tropical commodities which can not be produced in the temperate zone exceed a billion and a half dollars. We get every year in round figures, tropical sugar to the value of 400 millions of dollars; coffee, 240 millions of dollars; rubber, 180 millions; tobacco, 70 millions; coconuts, tea, and cacao about 30 millions each; bananas, 20 millions; and many other things in proportion.

The first four items in the list of our chief imports from all countries are silk, cane sugar, coffee, and rubber. The sugar production of Cuba alone is approximately equal to the entire sugar consumption of the United States.

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<sup>1</sup> Invitation paper read at the joint meeting of Section G (Botanical Sciences), American Association for the Advancement of Science, the Botanical Society of America, the American Phytopathological Society, and the American Society of Plant Physiologists at Kansas City, Mo., 29 December 1926.

Food supplies may be drawn from the Tropics to postpone to the distant future the time when food production will fail to keep pace with increasing population. The greatest contributions will be of sugar, oils, and fruits. Particularly large areas, now unused, are suitable for sugar cane, for coconuts and other oil palms, and for those most nutritious of fruits, the banana and the avocado.

The expanding culture of these and other food plants will bring up many new problems to the plant scientist for solution. The outstanding need in the Tropics today is for research. The extent to which they will be able to respond to calls for more food, fibres, rubber, etc., will depend upon the extent to which scientific methods are applied in their production. There are vast areas of unused land, in a climate where plants grow the year around, but at the present time, with some exceptions, agriculture is not highly developed. The principal crop plants are but little improved, and are grown, harvested, and handled by primitive methods; consequently the product of the unskilled labor of the tropical worker is barely sufficient to feed and clothe him and does not allow much of a surplus for export. We can depend on medical science to make the Tropics healthful, and on inventors and engineers to introduce in due time labor-saving machinery and effective means of transportation of the products to market by road, by rail, and by water, but the plant research needed is not yet started in any adequate manner.

Consider the vastness of the field. The area of tropical America is two and one-third times the area of the United States. It presents the most diverse conditions of climate, soil, and vegetation, so that the proper development of agriculture requires a large number of special investigations to cover the numerous crops in their local environments. The obvious problems needing scientific research are more numerous than in the United States; yet while we have over 300 stations for agricultural experimentation, there are in tropical America very few corresponding institutions, equipment, personnel, and financial support considered in the comparison.

This lack will not be met promptly by governments, and hope for support of research rests in large part on associations of producers or distributors of tropical products. Support of research by industrial corporations has become common in this



country, and we live in an age of marvels due to such research in chemistry, physics, and engineering, but it has not yet become so well understood that research in crop production is fundamental to continued prosperity.

The botanical problems awaiting solution cover the entire range of plant science. It is interesting to note that the principal tropical crops entering international commerce have been introduced from some other part of the world into the region where they are now grown. Sugar cane, which now dominates the West Indies, came from the Orient; rubber, native of the Amazon region, is grown principally in the far East; coffee, an African plant, is supplied to the world from Brazil; while cacao has made the reverse trip from South America to Africa. The Dutch East Indies are now growing the South American cinchona and the African oil palm. The banana and coconut, which we receive from the Caribbean regions, had their origin in the Pacific. We must conclude that the possibilities of plant introduction and acclimatization are by no means exhausted.

The improvement of tropical plants by modern methods of selection and breeding has barely begun. Such important crops as rubber, coffee, and cacao, and most of the tropical fruits, are still propagated by seeds. The perfection of methods of vegetative propagation and the replacement of existing cultures by special or named varieties would appear to be in the line of progress, and this opens up an interesting field of research in plant physiology on methods of propagation and the use of adapted stocks.

Plant diseases and insect pests are limiting factors in crop production in the Tropics. The culture of bananas has become impossible in places on account of the Panama disease. Budrot of coconuts has killed thousands of trees. The cacao industry of Ecuador seems to be threatened with disaster, owing to the spread of the witches'-broom disease. Sugar cane mosaic has cut into the profits of cane growers in many countries. The South American leaf disease of rubber may handicap the establishment of a plantation rubber industry in the home of *Hevea*. The destruction of the coffee industry of Ceylon and Java by leaf rust shows what may happen if this fungus should be brought to South America. In all these cases scientific investigations will require years of time, and should be begun before the critical need arrives.

Sugar cane problems are present in every country. In this period of intense world competition, sugar has become the cheapest food, and production of sugar cane at a profit depends on methods that will lower the cost of cane per ton. Cheap production in the Tropics has hitherto been based on the utilization of virgin lands, where cane grows almost without cultivation and without need for replanting for many years, but soon there will be no more forests to clear, and cane growing must pass from a pioneer condition of soil mining to a settled permanent agriculture. The plant specialist must reduce the hazard of crop losses by plant diseases and introduce new cane varieties locally adapted, disease resistant, with high tonnage yield, and richer in sugar.

Problems of maintenance of soil fertility in the older regions are already in evidence and will become pressing before research has provided solutions for questions of cover crops, green manures, legume inoculation, soil biology, and the fertilizer requirements of various soils and crops.

To a greater extent than at home there is need in the Tropics for breaking new ground in botanical research, to study methods of curing, fermentation, and extraction, and to search for useful by-products and new sources of oils, gums, latex, dyes, spices and fibres. Such investigations call for organized attack, for teamwork among botanists and with other branches of science. The plans should be well rounded, liberally supported, and carried on for many years. It may be predicted that there will be developed special crop stations maintained by the organized industries to supplement the work of governmental experiment stations, as exemplified by the Cuban Sugar Station of the Tropical Plant Research Foundation.

There is need in the case of each of the great tropical crop industries of a special survey to study the present conditions, to take an inventory of crop resources, point out and define the problems, diagnose the ills, and to outline a program of research, with recommendations as to equipment and personnel. Such a survey is now being undertaken by the Tropical Plant Research Foundation in the West Coast of Peru, in the interest of the sugar cane and cotton producers there.

*Rubber research needed.* We find ourselves now in a period of renewed interest in rubber production, when high prices have

led to a re-survey of the tropical world to find the most favorable location for new plantations. If rubber culture is to be successfully extended, much botanical research should be begun at once and liberally supported by the industry, for while much has been done on rubber, we have much yet to learn about the rubber tree and its requirements as to soil, location, and culture, the production of latex and its extraction, systems of tapping, bark renewal, and diseases associated with tapping. Most of the planted rubber trees are seedlings, and of course all the wild ones, yet it is said that there is a variation of 1000 per cent in the productivity of different trees. Consequently there is a field for the physiologist to investigate means by which the profitable trees may be selected and propagated vegetatively.

Rubber is subject to some serious diseases. A leaf disease in its native home has interfered with plantation enterprises, but Rands thinks it possible to select resistant types, and such attempts should not be delayed until the disease spreads to other centers of production. Immediate steps should be taken to select out these disease-resistant trees and propagate from them. If a new rubber industry is to be built up, it will be economy to spend money at the start to protect it from destructive future disease attacks. Every such crop should have its special experiment station, and before new areas are opened up for planting, the investors should arrange for preliminary investigations of an ecological nature. These should include studies of soils and climate, the character of the natural vegetation as indicators of soil and climatic conditions, and of diseases and pests likely to attack rubber trees.

*Botany and dietetics.* Another group of plant problems has been brought forward by medical workers, who tell us that an adequate supply of fresh, palatable, and vitamin-rich vegetable food is essential to the health of the people of tropical America. We think of these lands as producers of food in variety and profusion, yet the traveler notices at once the general lack of fresh vegetables, and is prepared to believe that great numbers of people live on the verge of deficiency diseases, with reduced vigor and disease resistance.

This is more than a gardener's problem. It is a field for botanical research. What are vitamins? How do they trace back to the effect of light on chlorophyll? In what way are

these mysterious activators of our metabolism related to plant growth? Under what conditions are they formed? What is the value from a vitamin standpoint of the various food plants and their products? This is known for very few tropical fruits and vegetables. We also lack sufficient information on the chemical composition of tropical foodstuffs to properly estimate their place in the dietary.

Production problems are also numerous. They include plant introduction, to enable us to profit from the experience of the older civilizations of China, India, and other countries; control of pests; seed supply; plant breeding and cultural studies to improve quality and to overcome the handicaps of rainy seasons and dry seasons.

It has been proposed that such a series of studies be undertaken in coöperation with medical workers, and it is urged that this is the next and most important move for public health in the Tropics.

*The botany of forestry.* There is much botanical work needed to lay a foundation for the agriculture and forestry of the future. At the present time most of the undeveloped areas of tropical America are covered by forests. The native farmers clear areas of a few acres by cutting down the trees and burning them. After growing a few crops they are likely to abandon the clearing and make a new one, thus gradually nibbling away the forest. But when North American or other foreign interests enter the field to grow sugar cane or bananas, they follow the same system of slashing down the native vegetation, but on tens of thousands of acres, salvaging only a portion of the more valuable woods and often buying their building material in the United States. So much of the Cuban forests have thus been converted into cane fields that that country can not meet its own timber requirements until a program of reforestation and forest management is adopted and carried out.

That tropical forests are burned, when the world needs all its wood, results primarily from lack of knowledge of tropical woods and their uses, which exposes the Tropics to an uneconomic competition from the highly organized lumber industry of the United States, which can export building material cheaper than the local tropical supply can be brought in. In the United States there are great mills with railroads and every mechanical or

engineering facility, operating in pure stands. In the Tropics are mixed forests of strange species, without roads, tools, or mills. Obviously it is easier and cheaper to import lumber into the Tropics.

But we have reached the time when the forests of the United States are inadequate to meet our own needs. Of the hardwoods needed for cabinet work, tool handles, and numerous industrial purposes, we have no longer enough, and even if we begin at once to replant hardwood forests in the United States, there will be a long gap between the exhaustion of the present supply and the availability of a new crop. Consequently for many years we shall have to supplement our shortage by importing tropical hardwoods or by wood substitutes. There is an abundant supply of splendid hardwoods in the Tropics, but it is not an easy matter for a lumberman to enter a tropical forest, with its mixture of species, many of which are unknown to the trade and are shunned because their virtues are not understood, and organize a modern logging operation.

Several years of botanical research are needed in each region that is to be developed. The first need is for naming the trees. Much taxonomic work has been done, but not enough. Let us express the hope that Latin names once given, and especially the old familiar names, will be allowed to stand. Foresters and others use Latin names for convenience of reference, and are impatient and seriously handicapped when required to learn a new set of names.

The investigators will be asked to discriminate closely between species that resemble each other and are often marketed under the same name, and also between varieties that differ in some quality of wood. They will be called upon also to discover means of identifying trees without fruit or flowers, and logs without foliage, by character of bark, structure, color, or other properties. In addition to naming the species in the forest, it is needful to know how many of each kind there are in a unit area, and for each important species its relation to soil, elevation, rainfall, and other ecological factors. The records taken should include dates of flowering and fruiting of all important tree species, including all occurring in quantity, even if of no present market value. Common names should be recorded, and local lore concerning the value or uses of the woods or plants.

The collections should include absolutely authentic wood specimens as well as ordinary herbarium material, and there should be duplicates enough to deposit sets of woods in the National Museum, the Yale Forest School, the U. S. Forest Products Laboratory, and the Field Museum, and herbarium material at least to the National Herbarium, the New York Botanical Garden, and the Gray Herbarium, with some for foreign exchanges. Without fail, sets of both botanical specimens and woods should be deposited in the proper institution in the country where collected.

The plant pathologist and the entomologist should be brought into the forest to survey for diseases and insects that are injuring the wood or preventing the reproduction of the species.

The next move will be to procure logs of species positively identified, for wood tests by the Forest Products Laboratory, to determine strength, stiffness, hardness, finish, and numerous other qualities essential to industrial use. The woods may then be introduced to manufacturers for practical factory tests, and steps taken to secure the granting of lumber concessions, erect permanent mills, build roads, and conduct logging operations on a basis of 15 to 20 or more year rotations.

The plant scientist has another service to render in the interest of complete utilization of the resources of these mixed tropical forests; that is to identify and work out methods for utilizing other forest products and by-products, such as oils, resins, waxes, gums; or latex products like rubber, balata, and chicle; tannins; nuts; medicinal plants like quinine; and pulp wood. There is need also for the physiological chemist to perfect methods of extraction or utilization of these products.

Some of these tropical forests will be cleared for agriculture. The remainder should be placed under forest management, and this, together with the reforestation of already devastated areas, introduces problems for botanical research additional to those of forest utilization just mentioned. These lie mainly in the field of ecology and plant physiology, and relate to problems of successions of vegetation after clearing, the effect of fires, studies of reproduction of each important species, their optimum requirements of rainfall, temperature, soil, and elevation, their reaction to shade, their production of seeds, the care of seedbeds, and young plantations, reproduction by cuttings, and similar

problems. Scarcely any of this fundamental knowledge is available for the tree species of tropical America.

These various botanical undertakings should be correlated with the work of geographers, climatologists, and soil scientists already in progress in tropical America. There would result in the end a vegetation map and report on natural resources of the American Tropics that would be of fundamental value as a basis for the development of this important part of the world.

It is not too large an order to be taken on by our botanical institutions working in coöperation. Much has been done that can be utilized. Much is in progress; for example, the notable study of the flora of the Caribbean countries that has been carried on for years by Dr. Britton and his associates and the botanists of Washington and Harvard. Neither is the problem of financial support insurmountable. I have sought to point out that our botanical research may have value as a business investment for organized industry.

There are various difficulties in the way. It is said to be hard to find trained specialists, but I have no doubt that the law of supply and demand functions here as elsewhere, and that when taxonomists and ecologists come to have a real market value, these waning subjects will be revived. The language question is very important. With our present outlook, inability to read and speak Spanish is a serious handicap.

Let us encourage students to prepare for research work in taxonomy and ecology in order that they may take part in the development of tropical forestry and agriculture that is surely coming. Let us also do what we can to assist the Latin American universities to build up their courses in the natural sciences and give training in forestry and agriculture, and, to round out the program, establish a graduate school of tropical agriculture or an effective international relation between existing institutions to make it possible for our men to secure tropical experience, and for Latin American students to complete their education in the United States.





# The nuclear phenomena and life history of *Urocystis Cepulae*<sup>1</sup>

ALPHEUS W. BLIZZARD

(WITH ONE TEXT FIGURE AND PLATES 1-4)

Although there have been in recent years many papers on various problems dealing with reproduction in the Ustilagineae, there is still a divergence of opinion concerning certain fundamental features in their nuclear history, especially as to the origin of the nuclei that fuse in the young spore; the significance of conidial conjugation; the number of nuclei in the cells of the hyphae of the parasitic mycelium.

The literature relating to the smuts has been summarized adequately by Fischer von Waldheim (21)<sup>2</sup> Brefeld (8) and Lutman (29). I shall refer only to such studies as bear more directly on these problems. As is well known, Prévost (37) in 1807 was the first to observe the germination of the spores of the smuts. The Tulasnes (45) in 1847 seem to have been the first to observe the conjugation of the conidia. De Bary (14, 17) Kühn (27) Fischer von Waldheim (21, 22) Brefeld (8, 9) and others have made studies of the life histories of the Ustilagineae and described a wide range of types of cell conjugation or anastomoses in the different genera and species of the group. Kühn (27) in 1857, in sections of the growing wheat sprouts, was the first to observe the infecting mycelial threads penetrating the host tissue. Kühn's observations were confirmed by Hoffman (24), de Bary (16), Wolff (51) Brefeld (9) and others. Brefeld's figures of the distribution of the smut hyphae in the tissue of the host in the case of *Ustilago Maydis* are perhaps the most adequate published so far for any smut.

The formation of the spores was studied by the Tulasnes (45, 46) de Bary (14, 17) Fischer von Waldheim (21) Wolff (51) Winter (50) Cornu (10) Woronin (52) and others.

De Bary (15) was the first to point out the analogy between the conjugation of the conidia of the smuts and the sexual fusions of the Conjugatae. Later (17, p. 196) he gave the following

<sup>1</sup> Contributions from the Department of Botany of Columbia University, no. 341.

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 112.

evidence to show why the conjugation of the conidia should be considered as having sexual significance:

First, the almost invariable occurrence of pairing under the normal conditions of germination; . . . Secondly, the great preponderance of union in pairs. The sporidia which are placed close to one another in whorls in *Tilletia*, *Entyloma*, *Urocystis*, and other genera unite, almost without exception, in pairs only, and when there is an odd sporidium, it usually does not conjugate, though its union with some pair might be easy, one might almost say would be very natural . . . These facts show that a change usually takes place in a pair after conjugation which renders a second union difficult or impossible, while it introduces the further development (18, p. 181).

Brefeld (8) on the other hand, considers the process of conjugation of the conidia as having no sexual significance whatever. He was the first to employ nutrient solutions successfully in prolonged cultures of the Ustilagineae, and observed the germination of a great many species. He reports bringing *Tilletia Caries* to the point of spore formation by his refined cultural methods. As a result of his intensive and tireless studies of these cultures he arrived at the following conclusion relative to the conjugation of the conidia: the fusing of the conidia never occurs so long as they are normally nourished and can multiply through budding; but it always takes place when the nutrient solution is exhausted (8, p. 50).

These differences of opinion of de Bary and Brefeld and their respective followers brought forth numerous theoretical discussions without furnishing new material to aid in solving the problem. With the development of modern cytological methods it became possible to study nuclear behavior in the Ustilagineae. The newer cytological results begin with the work of Dangeard (11) who observed nuclear fusion in the young spore.

According to this newer literature, we may consider as established the facts that the young spore cells, in the smuts so far studied, are binucleate, and on maturing become uninucleate by fusion, as maintained by Dangeard (11, 12) Harper (23) Maire (30) Lutman (29) Rawitscher (39, 41) and others. Furthermore there is unanimity of opinion that the mature uninucleate spore at time of germination produces a promycelium with uninucleate cells, in the case of the *Ustilago* group, and a multinucleate promycelium in the *Tilletia* group. In both the *Ustilago* and *Tilletia* groups the promycelia cut off uninucleate conidia, as maintained by Dangeard (11) Harper (23) Lutman (29) Rawitscher (39, 40) and Paravicini (34).

Relative to the origin of the two nuclei in the young spore, there is yet insufficient evidence to sustain any of the suggested theories. Lutman (29, p. 1208) says that in *Ustilago Zeae* and *U. levis*, "the vegetative mycelium composed of multinucleated cells, breaks up into short segments containing one or two nuclei which pass into spores." In *Entyloma Nymphaeae* he observed that the mycelial cells are binucleate but he did not work out their origin. Rawitscher (39) states that in *Ustilago Maydis* the binucleate condition arises by the fusion of two uninucleate hyphal cells at the time of spore formation. In *Ustilago Carbo*, Rawitscher thinks that the binucleate condition arises at the time of the conjugation of the conidia or the mycelial cells themselves. Rawitscher (40, 41) extended his observations to *Tilletia Tritici*, *Cintractia Montagnei*, and *Urocystis Violae*, each of which he finds corresponds in nuclear history to *Ustilago Carbo*. Paravicini (34) in 1917 confirms Rawitscher in general, as does Kniep (26) in 1921, studying the whole life cycle of *Urocystis Anemones* as grown on artificial media.

The interpretations of the conjugation of the conidia may be summarized as follows: (a) as having sexual significance: de Bary (17) and Federley (19), and as also initiating the binucleate condition: Rawitscher (39, 41) Paravicini (34) Kniep (26) Bauch (4, 5, 6); (b) as a non-sexual or vegetative process: Brefeld (8) Dangeard (11) Harper (13) Lutman (29) Dastur (13) and Sartoris (42).

Dangeard and Harper did not observe nuclear migration during conidial conjugation. Federley and Lutman observed the migration of the nucleus from one conidium to the other. Federley thought that nuclear fusion occurred immediately in the conidia after nuclear migration. Lutman was undecided on this point. On the other hand Rawitscher, Paravicini, Kniep, and Bauch state that nuclear fusion never occurs in the paired conidia, but that this pairing initiates the binucleate condition.

As to the number of nuclei in the mycelial cells there is likewise a difference of opinion. Fisch (20) Schmitz (43) and Dangeard (11) were of the opinion that the mycelial cells of the smuts were multinucleate. Lutman (29) says that the *Ustilago* group alone possesses multinucleate cells. On the other hand, Maire (30) and Lutman (29) observed binucleate mycelial cells in the *Tilletia* group. Rawitscher (39, 41) Paravicini (34) and Bauch

(4, 5) maintain that the mycelial cells may be either uninucleate or binucleate. Dastur (13) Noble (32) and Sartoris (42) state that the mycelial cells may be either uninucleate or multinucleate.

In 1921 Anderson (1) described the development and pathogenesis of the onion smut. Employing methods similar to those of Whitehead (49), he succeeded in germinating fresh spores in sterile nutrient media, and grew the mycelium in pure culture.

Anderson states that the germination of the spores begins with a protrusion of a short hemispherical promycelium from which a whorl of branches grows out. These branches grow indefinitely without producing conidia. The growing saprophytic mycelium breaks up into short plump cells which Anderson thinks have the function of conidia and are probably of great significance in the dissemination of the smut organism. Anderson figures the early infecting hyphae and describes the parasitic mycelium as intercellular and showing both uninucleate and binucleate cells. He also describes and figures large haustoria but adds that these absorbing organs are not common. Anderson made a cursory study of spore formation in which he contributed no new data.

Anderson and Osmun (3) reported in 1924, that they placed smutted leaves of the onion in damp soil in test tubes in which they remained for three years. At the expiration of that time they found that the spores germinated by placing them on agar plates. The authors state that the germination began, within 2 to 5 days, by developing a simple unbranched tube from the central spore. A globose promycelium is not produced. Usually the germ tube remained simple and unbranched for some time, then branched only sparingly. As to just why the smut spores did not germinate in the moist soil, their natural medium, in which they remained for years, the authors offered no explanation. It is reasonable to suppose that the tissue of the onion leaves, permitted to remain in damp soil for three years, would soften to such an extent that the smut spores would be freed in the soil. In such a case it is questionable if one can say with certainty that the spores recovered from the soil after a period of three years are those of *Urocystis Cepulae*.

The germination process and the non-production of conidia in *Urocystis Cepulae*, as described by Anderson in 1921, are in

direct opposition to the observations of Thaxter (47) who, in 1889, described the development of the onion smut fungus. Whitehead (49) in 1921 reported his work on the life history and morphology of the onion smut and confirmed Thaxter's observations in general.

My study of the life history and nuclear phenomena in *Urocystis Cepulae* has dealt primarily with the following points:

- (1) How and under what conditions do the spores germinate?
- (2) What is the nature of the saprophytic stage, and how is it initiated?
- (3) To grow the fungus in pure culture.
- (4) How is the fungus able to maintain itself in the soil?
- (5) To determine the nuclear phenomena of the saprophytic and parasitic mycelia.
- (6) The origin of the fertile and sterile cells that compose the spore ball, together with the nuclear phenomena in each.

#### MATERIAL AND METHODS

Since the onion smut is unfortunately not uncommon, an abundance of spores was available for experimental purposes. Dr. E. W. Olive, formerly of the Brooklyn Botanic Garden, and Dr. R. E. Kirby of Cornell University very kindly furnished me with a plentiful supply of spores for which I wish to express my sincere thanks.

Soil in a number of pots was infected with the smut spores. Onion seeds were sown at intervals so that seedlings at different stages of development were available at all times. The percentage of infection of the seedlings varied from about 30 to 100 per cent.

In order to study the parasitic stage of the organism, infected onion seedlings at various stages of development were fixed in different media. Flemming's weak chrom-osmic fixative gave the most satisfactory results. The material was imbedded in paraffin and sectioned three to five microns thick. The sections were stained with Flemming's triple and the iron-alum haematoxylin stains. The former proved to be the most useful. The septa between cells were brought out vividly, while the nucleoles, staining a brilliant red, served to distinguish the nuclei at all times. The Flemming triple stain was used with very short exposures and as little washing out as possible. The time and

the concentration of the stains were varied factors for the different stages of the life cycle of the organism

The spores were germinated in onion decoction, then plated out by the customary bacteriological method. The onion decoction was made by boiling 25 grams of sliced onion in one litre of distilled water until the liquid became opalescent. The decoction was then filtered, sterilized in the autoclave for 15 minutes at 15 pounds pressure, and the reaction adjusted to pH 7.1. The bean, carrot, potato, and onion media were prepared as follows: fresh green vegetables were washed thoroughly, tubed, and sterilized in the autoclave.

For the cytological study of the saprophytic mycelial cells and the germinating spores, a method proposed by Harper (23) was followed. Masses of mycelial cells were germinated in watch crystals, then, by means of a pipette, portions of the liquid containing the germinating mycelial cells were transferred into a drop of Flemming's solution. This fixing fluid containing the cells was 'stippled' on a slide previously covered with egg albumen. In the case of the germinating spores better success was obtained by coating a sterilized slide with a thin layer of onion agar; then placing spores from an unopened but mature smut pustule on the agar. The slide with the spores was then placed in a moist chamber. After several days the slide was stained in the usual way, the agar de-staining more rapidly than the fungus material. In this case, the iron-alum haematoxylin stain gave the best results.

#### SOIL INFECTION

Onion seeds were sown in soil which previously had been infected with spores of *Urocystis Cepulae*. In about eight to ten days onion seedlings appeared above the surface. Owing to the peculiar method of germination of the onion seedling, the 'knee' is the first portion of the seedling to come through the ground. This is caused by the rapid elongation of the descending limb of the cotyledon, together with the growth of the primary root, which forces the seed end of the cotyledon upward through the ground. Since the seed coats are still attached to the upper end of the cotyledon and are dragged out of the ground within ten to eighteen days, the seedling at this stage of development has a characteristic appearance, similar to that of the figure 7. Within three to four weeks the first leaf makes its appearance.

It was observed that the cotyledons are susceptible to the attack of the smut fungus during their growth through the soil, as has also been reported by Thaxter (47) Sirrine and Stewart (44) Walker and Jones (48) Whitehead (49) Anderson (1) and others. It is also common to find infected seedlings remaining beneath the surface for periods longer than normal. Upon sectioning these cotyledons they were found to be severely attacked by the fungus. The mycelium had already permeated the tissues, thus apparently retarding their growth.

The presence of the smut in the onion seedling is first indicated macroscopically by a slight thickening of the cotyledon which also appears darker in color. If the plant is held up to the light, little opaque blotches can be seen in the cotyledon. These dark areas are the developing spore pustules which will within three to four weeks burst through the host tissue, owing to the rapid development of the spores within the sorus, and the drying up of the host tissue. These infected spots make their appearance most often below the 'knee,' though they sometimes occur above it, that is, nearest the attached seed-coat. A number of centers of infection may occur on the same cotyledon. When the infections occur only between the seed coat and the 'knee,' or just below the 'knee,' the upper end of the cotyledon withers up and apparently cuts off any further advance of the fungus in the host tissue. In such cases, the plants when the leaves have appeared seem to have wholly recovered from the early infection. This is especially true of the leek. Several pots containing infected soil were sown with leek seed. About 80 per cent of the cotyledons of the leek seedlings became infected. Apparently all of the seedlings survived the attack of the smut and grew vigorously for weeks after the disappearance of the diseased cotyledon. On the other hand, a great many of the onion seedlings are killed by the fungus during the early stages of growth. If the infection is not too severe, however, and the fungus happens to invade the zone of meristematic tissue from which develop all of the future leaves, the infections become in a sense systemic.

Of all the sections that I have examined, not one instance was observed in which the fungus had invaded the root system. The cotyledons are the only portions of the onion seedlings which permit the entrance of the fungus.

## SPORE GERMINATION

Thaxter (47) in 1889 succeeded in inducing the spores of *Urocystis Cepulae* to germinate from material gathered in summer and kept until the following January. Some of the smutted onions were spread out in a shed and left for six months, after which they were placed in moistened earth, saturated with water, and allowed to freeze for a week or more. After this treatment some of the smut, removed to a warm room and kept moistened, germinated. Thaxter also germinated the spores in onion decoction. His cultures, he says, swarmed with bacteria which soon destroyed them. Whitehead (49) in 1921, by freezing the spore pustules, then washing them in mercuric-chloride solution, brought about germination in water and in onion juice. Anderson (1) in 1921 described a method by which fresh spores of this fungus were germinated under sterile conditions in onion decoction and various kinds of nutrient agar.

I succeeded in germinating fresh spores in onion decoction and on onion agar as follows:

*Spore germination in onion decoction.* Mature but unopened sori, preferably those infecting the upper end of the cotyledon, were selected, and sterilized in mercuric-chloride solution (1-1000) for five minutes. They were then rinsed thoroughly in sterile distilled water to remove all traces of the mercuric-chloride solution. The pustules while in the sterile water were cut into small pieces which were transferred to cover glasses containing a small drop of sterile onion decoction (25 grams of onion to 1000 cc. of distilled water). The cover slips with the spores were inverted over a Van Tieghem cell and sealed with lanolin. Some of the cultures were placed in the refrigerator at a temperature of 18° C. Other cultures were kept at room temperature about 22°-25° C. In those cultures in which germination occurred, about 3 to 5 per cent of the spores germinated within four to seven days. However, only a small percentage of the cultures prepared in this manner showed germination, which may be attributed to several causes: first, some of the sori may not have been sufficiently matured; second, the mercuric-chloride may have penetrated some pustules and killed the spores; third, there is a possibility that the sealed cells lacked the necessary amount of oxygen.

*Spore germination on onion agar.* The spore pustules were



sterilized and rinsed in water. The sterilized sori were transferred to sterile distilled water and the pustules crushed in order to scatter the spores in the water. Then the spores were transferred by means of a pipette to thin onion-agar plates, all glassware and implements being sterile. The plates were kept at a temperature of 18°-25° C. A much higher percentage of germination occurred on the onion agar plates than in onion decoction: often 70 to 80 per cent of the spores germinated. Some lots of spores taken from the same pustules germinated better on onion agar plates than in Van Tieghem cells in onion decoction. When the spores in onion decoction, sealed in Van Tieghem cells, had ceased to germinate, the following experiment was performed: the spores from two of the cultures in Van Tieghem cells were spread on onion agar plates. The other onion decoction cultures were permitted to remain sealed in the Van Tieghem cells, as controls. On the onion agar plates, about 80 per cent of all the spores so spread out germinated, while in the onion decoction cultures no further germination occurred, that is, only about five per cent of the spores had germinated, as described above.

All the spores do not germinate at the same time. At the end of the third day a few germ tubes were noticed. Additional spores began to germinate from day to day, up to and including the fifteenth day. This progressive germination is also evidenced by the development of the mycelial web about each spore. Some are visible to the naked eye, about one millimeter in diameter, while others appear as minute white dots. Examined microscopically the plate shows small tufts of mycelia, grading down to the spores just beginning to germinate. An agar plate from ten to fifteen days old presents spores in all stages of germination.

Anderson (*l. p.* 108) thinks that "the period of preparation for germination differs for different spores, so that the germination period extends over many months, possibly years."

Germination begins by the protrusion from the central spore of a somewhat spherical hyaline promycelial vesicle. At first this vesicle is about the size of the neighboring pseudospores of the spore ball. Growth takes place rapidly and its size increases until it may measure six to ten microns in diameter (FIG. 1, PLATE 3). This vesicle is apparently the promycelium. It varies considerably in size; in some cases it remains extremely small. Soon the sub-spherical promycelium buds out a tube,

which, from its subsequent development, we may characterize as a determinate hyphal branch (FIG. 1, PLATE 3). This is immediately followed in succession by other similar hyphae (FIGS. 2-4, PLATE 3). The number of branches that arise from the promycelium varies: four, six, and eight branches were observed in the greatest number of cases. Cross walls dividing these hyphae into cells soon appear (FIG. 3, PLATE 3). The cells nearest to the promycelium in turn bud off cells in a characteristic fashion indicated in FIGS. 3, 4, 5, PLATE 3. These hyphae develop immediately into mycelia of indeterminate capacity for growth which branch profusely so that within twelve to eighteen hours a dense web of mycelium is produced about the spores, either in onion decoction or on the onion agar plate. After two or three days, the older cells of the mycelium tend to round up and become more or less separated from each other: at the same time, rapid growth occurs at the periphery of the mycelial mat or ball.

Thaxter (47, p. 142) described the process of spore germination of *Urocystis Cepulae* as follows:

The germination consisted in the production from the central resting spore of usually a short hypha of germination, which commonly branched more or less and produced, terminally or laterally, small secondary spores, the so-called sporidia.

Whitehead (49) states that at the time of germination one, or rarely two germ tubes are produced, which cut off laterally minute oval sporidia. He says that these sporidia multiply by budding but do not conjugate. Anderson (1, p. 110) did not observe the production of conidia. He says, "No conidia have been observed on the promycelium or its branches or any where else throughout the development of the organism." My observations of spore germination of *Urocystis Cepulae* corroborate, in general, those of Anderson.

Since the spores of *U. Cepulae* do not germinate until the third to the seventh day, if the cultures are in the least contaminated, they are simply overrun with bacteria and molds before the germination of the spores begins. This is especially true when a nutrient solution of the nature of onion decoction is used. The use of pure cultures, as obtained by Anderson and myself, is imperative if the normal germination of the spores is to be observed.

#### NUCLEAR PHENOMENA DURING SPORE GERMINATION

Apparently heretofore the nuclear phenomena in the germina-

ting spores of *Urocystis Cepulae* have not been observed. In order to accomplish this, the following method was employed: spores were taken from unopened, mature, but sterilized sori and placed on sterile slides which previously had been coated with a thin layer of onion agar. The slides were then placed in a sterile moist chamber. After the spores began to germinate they were fixed *in situ* in Flemming's weak solution; then stained in the usual way in iron-alum haematoxylin stain. By this process the nuclei in the hyphal cells were clearly differentiated. The first divisions of the nucleus take place apparently in the spore, as has been described for *Urocystis Anemones* by Kniep (26), for *Tilletia Tritici* by Rawitscher (40, 41), and for *Urocystis Tritici* by Noble (32). Owing to the thick opaque spore wall and the surrounding envelope of sterile cells, the behavior of the nucleus within the germinating spore was not discernible even in thoroughly bleached preparations. In FIGS. 1 and 2, PLATE 3, the early stages of germination are represented. The nuclei pass from the spore through the short promycelium into each of the promycelial branches only after these structures have attained a certain development, for which reason neither the hyaline spherical promycelium nor the young hyphae as yet contain a nucleus. In slightly older stages as represented in FIG. 3, PLATE 3, the primary determinate hypha is already divided by a septum into two cells, each cell containing a nucleus measuring less than one micron in diameter. The cell next to the promycelium has begun to bud out another cell at its upper end. Hyphae are budded off by the promycelium in succession, as described above. As shown in FIGS. 4 and 5, PLATE 3, each hypha divides into cells, each of which contains a single nucleus. Each nucleus appears as a spherical body with chromatin content and a definite rounded nucleole. With the Flemming triple stain, the chromatin material was stained a light purple, and the nucleole a brilliant red.

Conjugation between the branches of the promycelium, as described by Kniep (26) for *Urocystis Anemones* and by Rawitscher (41) for *U. Violae*, was not observed in *U. Cepulae*. The primary hyphae continue to grow, and develop into uninucleate mycelial threads. Conidia, as noted above, are not produced in *U. Cepulae* in any stage of germination.

COMPARISON OF THE PROCESS OF GERMINATION AND THE ATTENDING NUCLEAR BEHAVIOR OF *UROCYSTIS CEPULAE* WITH THAT OF OTHER SPECIES OF *UROCYSTIS*

*Urocystis occulta* Wallr. Kühn (27) states that from two to three germ tubes of different sizes are formed. On the stronger promycelium two to six primary conidia appear. Wolff (51) in 1873 observed that the conidia germinate without falling off. According to Brefeld (9, p. 175, fig. 1, pl. 11) spores of this species produce a promycelium of varying length, which at its apex produces a whorl of four to six branches. These increase in length by apical growth. Both the promycelium and the branches become septate and poorer in protoplasmic content. Finally only the tips of the branches contain active protoplasm. Conidia, according to Brefeld, are never formed. Nutrient solutions did not change the form of this growth nor lead to the production of conidia. The description of McAlpine (31) in 1910 corresponds to that of Brefeld, with the exception that he calls the branches of the promycelium conidia.

*Urocystis Filipendulae* Tul. The whole germination process as described by Brefeld (9, p. 176, fig. 3, pl. 11) is quite similar to that observed by the writer for *U. Cepulae*: a very short promycelium with long branching mycelial outgrowths that produce no conidia.

*Urocystis Tritici* Koern. This species is distinguished from *U. occulta* only by the fact that the spores from the host of one will not infect the host of the other. The germination of the spores of *U. Tritici* has been described by McAlpine (31) and Noble (32). The latter states that the young promycelium contains a variable number of nuclei derived from the single nucleus of the spore. His figures show that two to four sporidia develop at the apex of the promycelium. One nucleus passes into each of the sporidia. The sporidia while attached to the promycelium produce slender germ-tubes which become binucleate by the migration and division of the single nucleus. In a few cases Noble observed the conjugation of conidia, and states "It appeared as if the single nucleus of each conjugating conidium migrated into the fusion germ tube" (p. 482).

*Urocystis Anemones* (Pers.) Winter. Fischer von Waldheim (21, 22) Plowright (35) Brefeld (9) Paravicini (34) and Kniep (26) have observed the germination of the spores of this species.

According to Brefeld a short thick promycelium develops which, in turn, produces three to four long mycelial threads (*p. 176, fig. 2, pl. 11*). Paravicini states that the branches of the promycelium fall off easily and by division produce a chain of uninucleate mycelial cells. The mycelial cells, he thinks, become binucleate by the dissolving of the cell wall separating two of these cells. However, Paravicini's figures do not convince one that this is the case. Kniep (26) reports growing saprophytically the complete life cycle from smut spore to smut spore of *U. Anemones* on 0.5 per cent malt extract media. He states that the fusion nucleus divides twice while in the spore. A short promycelium is developed which soon buds out a whorl of three or four branches. The nuclei now migrate from the spore into the promycelium. If but three branches are present, each of the nuclei passes into a respective branch, while the fourth nucleus remains in the stalk cell (promycelium). Conjugation occurs between these unicellular units so that two branches will contain two nuclei while the other two elements will be empty. The binucleate element increases in length by apical growth as described for *U. occulta*.

*Urocystis Violae* Sow. Prillieux (38) Dangeard (11) Brefeld (9) Paravicini (34) and Rawitscher (41) have all observed the germination of this species. Rawitscher states that at the time of germination of the spores, a multinucleate promycelial tube is formed, at the tip of which seven or eight conidia are produced. One nucleus migrates into each conidium. The conidia conjugate by slender conjugation canals through which the nucleus from one conidium migrates to the other. The binucleate conidia grow in length as Kniep observed for *U. Anemones*.

*Summarizing:* In *Urocystis occulta* and *U. Filipendulae* the process of germination, aside from the nuclear phenomena, which are still unknown, agrees in general with that of *U. Cepulae*. In *U. Anemones*, *U. Violae*, *U. Triticis*, and *U. Cepulae*, the fusion nucleus divides within the spore. The nuclei then migrate through the promycelium to the promycelial branches, into each of which one nucleus passes, forming uninucleate branches. In all of the above named six species of *Urocystis*, the promycelial branches germinate and grow in length while attached to the promycelium.

Conjugation of the promycelial branches (conidia) occurs in

*Urocystis Anemones*, *U. Violae*, and *U. Triticici*; while in *U. Cepulae* conjugation between promycelial branches does not take place, but the hyphae grow at once into a mycelium with uninucleate cells, without the production of conidia.

The promycelium of the species of this group varies in shape and size. It is sub-spherical in *U. Cepulae*, very short in *U. Filipendulae* and *U. Anemones*, tube-like in *U. occulta*, *U. Triticici*, and *U. Violae*.

#### ISOLATION AND CULTURAL CHARACTERISTICS OF THE MYCELIUM

Spores from mature pustules were spread on thin onion agar plates, after which the spores were located by means of the microscope and marked. In from ten to fifteen days the mycelium developed from a single spore will be visible to the unaided eye as a tiny white dot. These tiny white mycelial balls were transferred to various media. Luxuriant growths of mycelium occurred on onion agar, sterile bean, potato, carrot, and sterilized fresh onion.

*Cultures on onion agar.* A tiny white ball of mycelium which had developed from a single spore was transferred from the plate to an onion agar tube fourteen days after the spore had germinated. After forty-eight hours growth on the onion agar, it was photographed (FIG. 1, PLATE I). At this stage of growth it is a spherical mass of mycelium whose hyphal branches extend outward and radially in all directions, and is snow-white, due to the inclusion of air between the hyphae.

A growth of three and a half days is shown in FIG. 2, PLATE I. The structure of the mycelial weft and the growth habit of the hyphae is strikingly shown. At this stage it has a cottony appearance.

As the growth continues and the mycelial weft spreads more or less equally in all directions on the surface of the agar, simultaneously the mycelial threads pile up and intertwine so that after ten days' growth the culture appears as is shown in FIG. 3, PLATE I. The edges of the cultures are somewhat sharply marked, but with a hand lens one can detect a fringe of hyphae extending outward beyond the denser mass. Cultures at this age ranged in diameter from three to six millimeters and in height from one to two and a half millimeters. The surfaces of these cultures now show nodular or ridge-like elevations which

frequently arise at first in or about the center. Sometimes they occur over the entire surface of the culture. At this stage the cultures become a light gray.

After 16 days' growth the surface elevations have developed into pronounced wrinkles as indicated in FIG. 4, PLATE I. In this figure a delicate, compact, even fringe of hyphae is visible at the edges of the mass. Not all of the cultures of this age show such a pronounced fringe. The color has become darker with a gray sheen which is apparently due to the development of short aerial hyphae. FIG. 5, PLATE I, represents a growth of 18 days which had been transferred to onion agar from a culture growing on a bean. FIG. 6, PLATE I, represents a culture 61 days old. As growth proceeds the wrinkles become more like ridges with crests of higher elevation than in the preceding cultures. The gray changes to a brownish tinge. As the agar loses its water content the mycelium shows zonal growth more clearly.

*Cultures on sterilized potato.* The growth on fresh sterilized potato resembles that on onion agar, but is considerably more rank. After seven days' growth the culture measured  $6.5 \times 7$  mm. and 2.5 mm. thick. It is then a compact dense mat of mycelium, snow-white, with definite edges of irregular outline (FIG. 7, PLATE I). The surface is also irregular, suggesting the development of the wrinkles which will soon make their appearance. The culture continues to grow vigorously for about four weeks at which time a heavy rank growth covers the upper surface of the potato, showing the characteristic wrinkles described for the growth on onion agar. From this time on, the culture becomes less vigorous, seems to shrink together and dies within a few months. FIG. 8, PLATE I, shows a culture 51 days old. The mycelial mat is thin, dry, and quite flat. The crests of the remaining wrinkles crack open. The culture is white throughout.

The cause of the declining growth and ultimate death of the fungus is apparently due to some change in the potato. If transfers are placed on potatoes three months after sterilization, there occurs but a scanty growth, which soon dies.

*Cultures on sterilized onion.* Growth on sterilized onion at first was exceedingly slow. Instead of spreading, as was the case on onion agar and on potato, the mycelium developed a tiny compact ball about one millimeter in diameter, hygrophanous, and gray. A few mycelial threads extending from the

ball start other centers of growth, each center subsequently rounding up into a little sphere, similar to the first. After a growth of two weeks a number of these little mycelial balls were present in the immediate vicinity of the original transfer. As evaporation of the water goes on the mycelial balls increase in size, pile up over each other, and finally coalesce in such a manner that a mycelial mat with uneven and wrinkled surface is formed. The crests of the higher elevations become white while the mass of the mycelium remains hygrophanous and gray (FIG. 9, PLATE I).

An attempt was made to induce spore formation on a living onion bulb. An onion was pared under aseptic conditions and infected with the fungus, then placed in a sterile moist chamber. In a few days the fungus showed signs of growth. Simultaneously the onion tissue began to darken in the immediate vicinity of the infection, as if the tissue were being killed. Finally the fungus produced the characteristic mycelial mat with wrinkled surface (FIG. 10, PLATE I). At no time was there any appearance of spore formation.

*Cultures on sterilized bean pods.* Transfers were made from the onion agar plate to sterilized bean pods. Growth was vigorous from the beginning. At first the culture shows a snow-white web of mycelium, as observed on the onion agar and on the potato. In about a week it appears as a compact mat of mycelium with definite but irregular edges. The growth on the bean distinguishes itself from that on other media by the fact that the fungus tends to pile high in a mass, layer upon layer, as shown in FIG. 1, PLATE 2, which shows a seventeen day old culture. As growth progresses the culture extends itself partly around the bean, as indicated in the 51 day old culture in FIG. 2, PLATE 2. At this stage the wrinkles are larger and coarser, a specific feature of the bean cultures. The central mass of the culture ranges from dark gray to brown, while in the periphery a delicate white web of mycelium is evident. Zonal growth next appears, a certain indication of the appearance of conditions unfavorable for the fungus. Secondary mycelial mats often occur, started probably by bits of hyphae from the primary mat being scattered in handling the culture.

In no case has the writer ever observed conidia or spore formation of any kind on culture media.



*Cultures on sterilized carrot.* Growth on the carrot resembles that on the sterilized onion. After the carrot becomes drier the fungus takes on the characteristic form of a mycelial mat. FIG. 4, PLATE 2 represents a 25 day growth. Soon little rope-like ridges appear, which anastomose as indicated in FIG. 5, PLATE 2, a 47 day old culture. As the culture ages, the ridges become proportionately enlarged and run together, thus producing a more even surface than is seen on the onion agar, potato, or bean cultures (FIG. 6, PLATE 2).

*Cultures on liquid nutrient media.* Bits of the mycelium were transferred to tubes of 2 per cent cane sugar solution, and tubes containing onion decoction. The growth was similar in each case. The mycelium formed little balls which never exceeded more than one to two millimeters in diameter. If the mycelial web becomes attached to the sides of the test tube a slight growth of white mycelium results.

In résumé it can be said that very young cultures of *Urocystis Cepulae*, on solid media, present a cottony appearance. In older cultures when the water content is not too high, the wrinkling of the surface is a constant feature. The shape and form of the wrinkles vary according to the kind of media used and the age of the culture. FIG. 7, PLATE 1, and FIG. 1, PLATE 2, represent cultures of the same age but grown on different media, showing characteristic variations in appearance. FIG. 6, PLATE 1, and FIGS. 2 and 6, PLATE 2, are cultures of different ages grown on agar, bean, and carrot, respectively, and likewise show that the wrinkles vary in form, size, and direction of growth. In a liquid medium the culture is characterized by the formation of small mycelial balls.

The vitality of the fungus is not reduced by transfers from one culture to another, or, so far as tested, from one kind of medium to another. Apparently cultures can be continued indefinitely. FIG. 5, PLATE 1, shows a culture which was transferred from bean to onion agar, this representing the fourth transfer from the agar plate. FIG. 1, PLATE 2 was transferred from carrot to bean, and likewise represents a fourth transfer.

The growth habit of *Urocystis Cepulae* in culture is quite distinctive in appearance when compared with cultures of certain other fungi. Blakeslee's photographs of *Mucor Mucedo* (7, fig. 55) and of *Phycomyces nitens* (fig. 52) show that the hyphae

spread out evenly over the medium, forming a loose mycelial web on the surface of the plate, with an abundance of aerial hyphae. On the other hand cultures of yeast obtained by Lindner (28) are quite similar in appearance to those of *U. Cepulae*. Certain of Lindner's cultures (numbers 68, 70, plate 85) show that the yeast cells pile up on each other and spread out, forming a thick mat with a definite wrinkled surface, comparable in appearance to my FIG. 4, PLATE 1, and FIG. 6, PLATE 2 of *U. Cepulae*.

The cultural characteristics of *Tilletia Tritici* as shown by Sartoris (42, figs. 2, 4, plate 39) are different from those of *Urocystis Cepulae*. Sartoris says

Two or three weeks after germination of the chlamydospores on oatmeal agar a mycelial layer appears. This spreads until it covers the surface of the agar by its radial growth. It is white at the surface of the layer and of a fine leathery texture. It is about 0.5 to 1 cm. in thickness at the center of the mat: here also it is thickest and forms a peak (p. 625).

The mycelium of *Ustilago Zeae* grown on glycerin agar by Sartoris forms a thin mycelial mat. A study of his photograph (fig. 3, plate 39), gives the impression of nodular elevations formed on the surface, but quite different in appearance from the cultures of *U. Cepulae*.

Potter (36) grew in pure culture the mycelium of *Sorosporium Reilianum*, the causal organism of head-smut of sorghum and maize. He found that the organism developed well on malt-extract, beerwort, and carrot agars. A synthetic dextrose agar also proved to be a favorable medium. The growth on carrot agar at 30° C. produced a thick mycelial mat whose surface bore numerous wrinkles that anastomosed freely (fig. 1, plate 34). His figs. 2, 3, plate 34 on glucose and on carrot agar respectively Potter states, "show the characteristic rugose growth." Thus the cultural characteristics of *Sorosporium Reilianum* are indistinguishable in appearance from those of *Urocystis Cepulae*.

Anderson (1) grew *U. Cepulae* on a large number of culture media, and describes its growth on onion agar as follows:

The growth begins with a dense white felt . . . after about a week wrinkles begin to appear near the center, and these spread and become sharper and the irregular ridges more elevated with age. . . . The crests of the ridges become hygrophanous and gray (p. 113).

My observations of the cultural characteristics of *U. Cepulae* in general corroborate those of Anderson. Anderson states that

"the convoluted gray growth on onion agar is perhaps the best diagnostic cultural character of the species" (p. 114). Certainly this is true when cultures of *U. Cepulae* are compared with cultures of fungi like the Mucorineae. But it would be difficult or almost impossible to distinguish cultures of *U. Cepulae* from certain yeast cultures or cultures of *Sorosporium Reilianum*, as noted above.

#### THE NUCLEAR PHENOMENA IN THE SAPROPHYTIC MYCELIUM

In order to study the nuclear phenomena in the young mycelium, spores were placed on a slide previously coated with agar, and permitted to germinate under sterile conditions. In ten to fifteen days little tufts of mycelia in various stages of growth developed about the spores. These little tufts of mycelia were fixed *in situ* in Flemming's weak solution and stained in iron-alum haematoxylin and Flemming's triple stain.

The microscopic appearance of the mycelium varies with the age of the cultures. In examining the little tufts of mycelium on the onion agar, mycelial threads were observed to have spread out over the surface of the agar to a distance of about 100 microns from the periphery of the culture mass. In some of these hyphal threads the basal cells soon become empty while the tips are full of dense homogeneous protoplasm. It is difficult in many cases to find any connection between the hyphal tips thus isolated and the main culture. These isolated hyphal tips branch and become the center of new growths which soon fuse with the older mass.

These young cultures are composed of slender branching hyphae measuring from one to two microns in diameter, with rather homogeneous cytoplasm. The hyphae, in fact, do not differ materially from the hyphae as described at the time of spore germination, represented by figs. 4 and 5, PLATE 3. In all young cultures thus examined, the mycelium was observed to be composed of uninucleate cells. The nuclei are definitely differentiated by the stains, each nucleus containing a well-defined nucleolus.

As noted, conidia are not produced by any part of the young mycelium. Paravicini (34) reports in the case of *Urocystis Anemones*, which species he says produces no conidia, that nuclear migration from one hyphal cell to an adjacent cell of

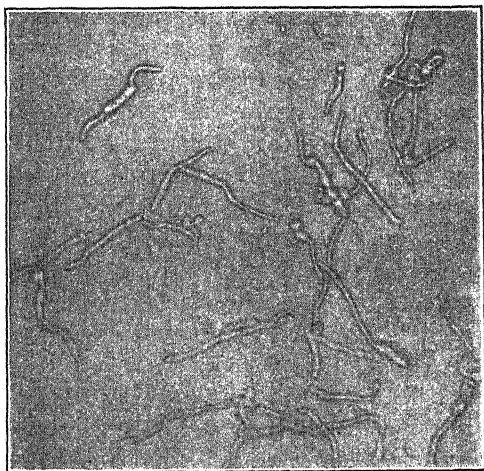
the same hypha may occur. This nuclear migration, according to Paravicini, is made possible by the gelatinization of the septum between the two cells. Thus a binucleate condition is initiated in the hyphae, which he thinks is the origin of the binucleate condition that is found at spore formation. A very careful examination of the saprophytic mycelial cells clearly shows that no such nuclear migration occurs in *Urocystis Cepulae*. The cells of the saprophytic mycelium are uninucleate from the beginning and remain so throughout their existence.

For the study of older mycelia two methods were employed: (1) mats of mycelia growing on agar were sectioned and stained; (2) small portions of mycelia were broken up in water and stippled on the slide.

Small blocks of agar containing the eighteen to twenty day old mycelium were fixed and imbedded in paraffin. These blocks were sectioned 3-5 microns thick, and stained with Flemming's triple and iron-alum haematoxylin stains. In such sections it could be clearly observed that the hyphae of the older cultures presented a different appearance from that of younger growths. Some of the hyphal threads that had penetrated the agar could be traced continuously into the aerial hyphae in the center of the culture mass. These hyphae were considerably larger than the surrounding hyphae, measuring 2-3.5 microns in diameter (FIG. 9, PLATE 3). Their cells appear well nourished and the cytoplasm contains refractive granules and stains densely (FIG. 7, PLATE 3). In the same section can be seen other hyphae whose cells tend to be rounded at the ends and constricted in the middle, giving the entire hyphal thread the appearance of a chain of beads. Some of these dumb-bell shaped cells are rich in cytoplasm (FIG. 6, PLATE 3). In the very old or more desiccated cultures the cytoplasm was reduced to a primordial utricle with thin strands of cytoplasm stretching across the cell from side to side (FIG. 10, PLATE 3). On either side of the nucleus of these cells, towards the end, are large vacuoles. In each case, the nucleus occupied the central portion of the cell in the region of the constriction, surrounded by a scanty amount of cytoplasm.

The method of branching of the hyphal cells is characteristic. Most frequently at the distal end of the cell just beneath the septum, a cytoplasmic extrusion occurs (FIG. 12, PLATE 3).

This growth may continue until it is almost or quite the length of the mother cell, before a nucleus appears in it (FIG. 4, PLATE 3). In some cases the nucleus migrates into the branch and there divides (FIG. 9, PLATE 3). In other instances two nuclei were observed in the mother cell and none in the branch. Finally a nucleus is present in each portion, and a septum is laid down at or near the base of the branch. The axis of growth of the branching cell forms an angle of about  $70^{\circ}$  with the long axis of the mother cell, and gives to the mycelium a characteristic appearance. The branches may at times be given off at the lower end of the cell as shown in FIG. 12, PLATE 3.



TEXT-FIG. 1. A photomicrograph of germinating hyphal cells in onion decoction culture 18 hours. Each cell has produced a thin germ tube at one or both ends.  $\times 600$ .

It was observed that any tiny piece of mycelium, even the individual cells of the mycelium themselves, are capable of starting new growths on the various media. Bits of mycelium from the cultures on bean, on carrot, and on onion, were transferred to a few drops of sterile water in a hollow ground slide. The mycelium was stirred and shaken in such a manner as to separate the cells from each other. The individual cells appeared as represented in FIG. 6, PLATE 3. These cells were then spread on thin agar plates. With the aid of the microscope individual cells were located on the onion agar and marked so

that their subsequent development could be followed. Every cell observed 'germinated' (see TEXT-FIGURE 1), and produced within fifteen to eighteen days a mycelial growth visible to the unaided eye, in a manner similar to that described above for the germinating spore. The mycelia resulting from such germinating hyphal cells are shown in FIG. 7, PLATE 2. For comparison, FIG. 8, PLATE 2 shows mycelium which has developed directly from a mass of germinating spores. The general appearance of the mycelia resulting from germinating hyphal cells, and that of the mycelia developing from a mass of germinating spores is the same.

In another experiment, bits of mycelia were broken up into their cellular elements, as described above. Then by means of a sterile pipette these hyphal cells were sprayed over a sterilized bean pod. Apparently wherever a mycelial cell settled upon the bean pod a growth resulted as evidenced by the distribution of the hyphal mats shown in FIG. 3, PLATE 2. This culture was 51 days old at the time of photographing. A number of the mycelial colonies have fused. On the upper portion of the bean pod a well developed mycelial mat with the characteristic wrinkled surface is formed.

In order to follow the nuclear phenomena in germinating hyphal cells such as are shown in TEXT-FIGURE 1, hyphal cells broken apart were placed in a hanging drop of onion decoction and sealed in a Van Tieghem cell. The usual precautions to keep the culture pure were observed. After eighteen hours a drop of Flemming's weak solution was mixed with the culture and the preparation was allowed to stand for thirty minutes. The fixing solution, together with the mycelial cells, was then stippled on slides which had previously been coated with egg albumin. The slides were then placed in 50 and 70 per cent alcohol to coagulate the albumin. By this method numerous hyphal cells remained attached to each slide and were subsequently stained by Flemming's triple and iron-alum haematoxylin stains. In all the slides examined apparently every one of the dumb-bell shaped cells 'germinated' by budding out at one end a slender cytoplasmic tube (FIGS. 8, 11, 14, PLATE 3). The nucleus soon migrates into the tube and divides as indicated in the upper figure of FIG. 8, PLATE 3. A septum is laid down between the two nuclei as indicated in the lower figure of FIG.

8, PLATE 3. Some of the cells may bud out slender hyphal tubes at both ends. In these cases, the nucleus still remains in the original cell (FIG. 14, PLATE 3). In other cases, the nucleus and cytoplasm appear to migrate into the germ tube (FIG. 8, PLATE 3) leaving the original hyphal cell empty. Subsequent branching of the hypha takes place, as previously described. The mycelium arising from these hyphal cells is composed of uninucleate cells, as is the mycelium which had its origin from a germinating spore.

Apparently each mycelial cell of the saprophytic mycelium may function as an oidial spore, serving to propagate and disseminate the fungus in the soil.

Dessication does not kill the mycelial cells, as is indicated by the following experiment. Cultures which had been growing on thin agar plates were permitted to dry. After four weeks, when the plates were air dry, portions of the mycelia were examined and observed to be composed of hyphal threads which had the appearance of oidial chains, due to the rounding of the cells at their ends and a constriction in the middle (FIG. 10, PLATE 3). Such cells measured about  $6 \times 1.5$ –2 microns. Some of this dessicated mycelium was shaken in water to separate the cellular elements. These separated, dessicated mycelial cells were placed in onion decoction and other media favorable to their growth. In eighteen to twenty-four hours they budded out slender germ tubes, as described above. From such dessicated cells, cultures were developed similar to the one which is represented in FIG. 7, PLATE 2.

The persistence of the fungus in the soil was shown by the following experiment: Pots containing infected soil in which onion seedlings infected with the smut fungus had previously been grown, were set aside in March, 1921, in a dry room, and exposed to the sun. This soil was 'air-dry' throughout the summer of 1921 until November of that year, at which time the soil was moistened and sown with onion seeds. In a few weeks about 60 per cent of the seedlings showed smut infection. Controls showed no infection.

#### INFECTION OF THE HOST WITH MYCELIA FROM CULTURES

To test the ability of the saprophytic mycelium to infect the natural host, the following experiments were made:

*Experiment A.* Soil was placed in six large test tubes which

were then plugged with cotton and sterilized in the autoclave for thirty minutes under eighteen pounds pressure. Onion seeds were sterilized for five to ten minutes in mercuric-chloride solution, then washed in sterile water and placed in a moist sterile chamber. When the onion seeds germinated, healthy vigorous onion seedlings at different stages of development were transferred to the sterile soil in three of the test tubes, numbered one to three. To these three test tubes were transferred mycelium from the various cultures, in each instance packing it around the seedlings as closely as possible. The other three test tubes were kept as controls, some sterilized onion seeds having been planted in each of them. Results are shown in TABLE I.

TABLE I

<i>No. of test tube</i>	<i>No. of seedlings</i>	<i>Infected</i>	<i>Not infected</i>	<i>Controls</i>
1	12	12	0	0
2	12	3	9	0
3	12	6	6	0
	<hr/>	<hr/>	<hr/>	<hr/>
Total	36	21	15	0

*Experiment B.* Soil was placed in pots and sterilized by dry heat for four hours at a temperature of 178° C. Ten onion seedlings were removed from the sterile moist chamber to each pot. The onion seedlings were then covered with bits of mycelium which had been broken up in sterile water and a half-inch layer of sterile soil placed over the seedlings in the pot. This top layer was then thoroughly moistened with sterile water in which a considerable amount of the fungus mycelium had been placed. Sterile water was used to keep the soil moist. Pieces of glass were placed over the top of each pot. Controls were run with each. The results are shown in TABLE 2.

TABLE 2

<i>Pot</i>	<i>No. of seedlings</i>	<i>Infected</i>	<i>Not infected</i>	<i>Controls</i>
1	10	7	3	0
2	10	10	0	0
3	10	3	7	0
4	10	2	8	0
5	10	10	0	0
6	10	5	5	0
	<hr/>	<hr/>	<hr/>	<hr/>
Total	60	37	23	0



Results of the two experiments:

	<i>Infected</i>	<i>Not infected</i>
6 test tubes	21	15
6 pots	37	23
	60.5% infected.	

The possibility that smut spores were carried over with the inoculum is hardly conceivable when one considers that most of the cultures from which the mycelium was taken were each a product of a single spore. Furthermore, some of the inoculum was mycelium from the second and third transfer from the original spore or spores that produced the mycelium. It is interesting to note here that some of the mycelium that was placed in the soil in *Pot 2* was recovered after twelve hours. This mycelium was mounted on a slide in the usual way and stained. Some of the cells had begun to germinate and are represented in FIG. 13, PLATE 3.

It seems probable that infection in the field may commonly take place from mycelia or hyphal cells, rather than more directly from the germinating spore.

In the above experiments it was observed that the period elapsing between the time when the young seedlings were inoculated with mycelial cells and the first appearance of the dark pustules varied from nine to fourteen days in the case of the young cotyledons. Seven days later the mature pustules were formed. Thus the parasitic stage of the onion smut, in cases when the seedling only is infected, may cover a comparatively short period from the time of infection until complete spore formation, that is, from three to four weeks. In the case of systemic infections, this period of time is considerably lengthened, the pustules not making their appearance until the leaves are well-developed.

#### THE CHARACTER AND NUCLEAR PHENOMENA OF THE PARASITIC MYCELIUM

The parasitic mycelium of *Urocystis Cepulae* is intercellular throughout its existence. The vegetative hyphae can be observed to run singly between the cells of the host, or there may be three or four mycelial threads running parallel in the intercellular spaces. These hyphae are delicate slender tubes, most of the cells of which are long and slim, measuring 20-25 microns in

length, to 1-1.5 microns in diameter. They are most frequently observed running parallel to the long axis of the cells of the host tissue. As one follows these hyphae up and down, nests of mycelia are found which have been formed by frequent branching of the hypha. With Flemming's triple stain the cellular boundaries are well brought out. Branches of the hyphal cells arise usually at the end, and just beneath the septum (FIG. 4, PLATE 3) as described for the saprophytic hyphae. The cytoplasm is homogeneous. The nuclei are extremely small structures throughout the parasitic stage of *U. Cepulae*, averaging in size from 0.5 to 0.8 microns in diameter, while the nucleoles, of course, are much smaller. By carefully manipulating the Flemming triple stain the whole nucleus will stain a light purple while the nucleole will be a brilliant red, the cell septa staining a dull red. However, in most cases, because of the affinity of some of the cells for the gentian violet, de-staining is advisable until only the nucleoles and cell septa stand out prominently.

Certain of the hyphal cells were observed to contain but one nucleus (FIGS. 4, 5, PLATE 4). These uninucleate cells were always observed to be at some distance from the young developing sorus. I have followed with extreme care the tortuous route of these hyphae as they wind their way over and around the host cells, but in spite of diligent search I was unable to find any trace of cell fusion. Haustoria or special feeding branches do not occur in *U. Cepulae*: In one case, two clavate hyphae were observed to have penetrated the cell wall and extended some distance within the cell. In one other case, an unusually large hypha was observed to have penetrated through one cell, and apparently was making its way into the adjoining host cell. Such cases are unusual and are to be considered as variations from the usual growth habit of the organism. Whitehead (49) observed hyphae of a simple type, which he called haustoria, within the host cells, and says that they are similar in appearance to the intercellular hyphae. On the other hand, Anderson (1) has described and figured 'large complicated haustoria.' He adds, however, that "in some infections none could be found, while in others they are fairly common." (p. 126). Neither Whitehead nor the writer observed such complex haustorial structures as reported by Anderson.

As one traces the mycelium towards a sorus, the hyphae

running in the intercellular spaces increase in number by profuse branching; they may twine around each other forming strands of mycelium which force the host cells farther apart. The cells of these hyphae are predominantly binucleate. The two nuclei lie in the center of the cell a short distance apart (FIGS. 1-3, PLATE 4). Numerous transverse branches from the mycelial strands penetrate radially the spaces between the mesophyll cells of the host. The vascular bundles are never invaded by the fungus. In the region where the sorus develops, the host tissue is thoroughly infiltrated with the hyphae of the parasite. The hyphal cells of the sorus primordia are all binucleate. This observation agrees with those of Lutman (29) for *Urocystis Anemones*.

Thus the hyphal cells of the parasitic mycelium are at first uninucleate but become binucleated as they approach the sorus primordium where all the cells of the sporogenous hyphae are binucleate (FIGS. 6, 7, PLATE 4). These sporogenous regions constitute the great bulk of the parasitic mycelium and thus it follows that the great mass of the parasitic hyphal cells of *Urocystis Cepulae* are binucleate. Lutman (29, p. 1213) reports in the case of *U. Anemones* that the hyphal cells in the host tissue are binucleate. He says, "whether this is true of all the cells it is impossible to say, but certainly a majority of the hyphal cells show two nuclei closely associated."

The binucleate cell condition in *Urocystis Cepulae* does not seem to arise at any one point in the mycelium. I am confident that cell fusions do not occur, as Rawitscher (39) described for *Ustilago Maydis*, at the time of spore formation. In all the numerous preparations of the parasitic mycelia that were examined, not one case of cell fusion between uninucleate mycelial cells was observed. Clamp connections such as were reported by Osner (33, p. 212) for *Ustilago striaeformis* are not present in *Urocystis Cepulae*.

The nuclear phenomena in *U. Cepulae* agree in the main with those of *Doassansia Sagittariae* as described by Rawitscher (41) in 1922, when he states that the binucleate condition arises a little before spore formation.

#### SPORE AND SPORE-BALL FORMATION

Kühn in 1858 was the first to describe spore formation in

the genus *Urocystis*. Spore formation has been described for the following species: *Urocystis occulta*, Kühn (27) 1858; Wolfe (51) 1873; *U. Colchici*, Winter (50) 1876; *U. Violae*, Prillieux (38) 1880, Dangeard (11) 1894, Paravicini (34) 1917, Rawitscher (41) 1922; *U. Anemones*, Lutman (29) 1910, Paravicini (34) 1917; *U. Cepulae*, Thaxter (47) 1889, Whitehead (49) 1921, Anderson (1) 1921.

Prillieux (38) states that the sporogenous filaments form the fertile spores acrogenously, and agrees with de Bary (16, p. 125) 1866, that the sterile cells are formed by short hyphae which fix themselves to the surface of the young spore. Dangeard (11) in 1892 observed that the young spore cell contains two nuclei which fuse, and states that the sterile cells contain no nuclei. He thinks that "elles servent de nourriture aux cellules fertiles" (p. 261).

In *Urocystis Anemones*, Lutman (29) states that the fertile spores, together with the pseudo-spores, develop directly from binucleate cells. "Those that will become sterile contain little stainable material while the cytoplasm of the fertile spore is quite dense," (p. 1214). Lutman agrees with Dangeard that the two nuclei in the fertile cell fuse into one. Paravicini (34) and Rawitscher (41) in general confirm Dangeard's and Lutman's observations.

*Spore formation in Urocystis Cepulae.* In *U. Cepulae* the hyphae of the strands of mycelium crowded between the cells of the host plant in the sorus primordium finally begin to curve and coil in a characteristic manner (FIGS. 6, 7, PLATE 4). This feature, and also the increased tendency of the cytoplasm to take the stain, are characteristics which serve to distinguish the first differentiation of the young sorus. A study of these sporogenous hyphae shows abundant cases of the early stages of the developing spore balls.

Success in staining these stages requires rather thin sections (three microns thick), and the proper concentration of the gentian violet and orange G stains. These concentrations were so manipulated that the nuclei and cell septa were distinguishable at all stages.

The spore ball has its beginning with the production of short hyphal branches which may be designated as sporogenous branches. These sporogenous branches form a sort of corymbose

branching system, each branch of which consists of short cells that are binucleate. The spore ball primordia are differentiated from the surrounding hyphae in the sorus by their more densely stained cell cytoplasm, and a decided curving of the hyphal branches, even before the central fertile cell of the individual spore ball is distinguishable. In a single young sorus great numbers of spore balls can be observed in all stages of development.

The definitive fertile cell differentiates itself from the cells of the same branch and the cells of other adjacent sporogenous hyphae by a notable increase in size. In FIG. 6, PLATE 4, the central cell is shown slightly larger than the cells of the surrounding hyphae. All the cells are plainly binucleate. In sections of this thinness all the branches of a single spore ball are not included. The spore ball primordium measures about 10 microns across, the hyphal cells about 1.5-2 microns. In FIGS. 7 and 8, PLATE 4, the central cell has increased perceptibly in size.

At this stage of development careful study was given to determine whether cell fusions of any sort occur. Since the sporogenous branches curve back on themselves, one may get the impression that some form of cell fusion may be taking place, but such is not the case. The young fertile spore cell takes its origin from a binucleate centrally located cell of a sporogenous branch. The rapid development of the fertile cell results in crowding the adjacent cells as indicated in FIG. 9, PLATE 4, where the two ends of the original sporogenous branch are still recognizable and attached to the young spore cell.

The pseudo-spore primordia, as well as the central fertile cell, are at first binucleate (FIGS. 6-10, PLATE 4). This observation agrees with those of Lutman (29) and Paravicini (34) for *U. Anemones* and Rawitscher (41) for *U. Violae*. In FIG. 10, PLATE 4, the only apparent difference in the cells of the sporogenous branches is in the shape and size of the fertile spore cell as compared with the pseudo-spore primordia, the cells of which have not increased in size perceptibly. From this time on, the fast growing central cell tends to suppress the surrounding cells of the sporogenous hyphal branches. The contents of the surrounding cells, as Dangeard (11) holds, apparently serve as food supply for the developing fertile cell. It seems

probable that all of these binucleate cells of the young spore ball are potential spores. One of them begins to grow at the expense of the others, because it happens, very likely, to be in a more favorable position for food supply. As the central cell enlarges, the surrounding hyphal cells—the primordia of the pseudo-spores—become appressed to its surface. In FIG. 10, PLATE 4, the central cell is still binucleate and shows its attachment to the original sporogenous branch, while the primordial cells of the pseudo-spores have not increased perceptibly in size.

FIGS. 8–16, PLATE 4 are of the same magnifications, with the exception of FIG. 10, and serve to give an accurate representation of the rate of growth of the central fertile cell as compared with the growth of the pseudo-spores.

When the fertile spore cell has increased in size to about four microns in diameter, the two nuclei meet near the center of the cell and fuse (FIG. 11, PLATE 4). The stage at which fusion occurs agrees with that described by Dangeard (11) and by Rawitscher (41) for *U. Violae* and that described by Lutman (29) and by Paracivini (34) for *U. Anemones*. Because of the minuteness of the nuclei, details of the nuclear fusion could not be worked out.

With the stage of the nuclear fusion in the central cell, the pseudo-spores have increased in size and their two nuclei, in some cases, are pressed together (FIG. 12) giving the pseudo-spores the appearance of uninucleate cells. In FIG. 13, PLATE 4, the young spore measures 9.5 microns in diameter, the fusion nucleus one micron, while the whole spore ball is 12 microns across. In FIG. 14, PLATE 4, the fertile cell is surrounded by a darkly stained substance which is the first indication of the developing spore wall. The spore-wall substance is evidently formed from the fertile spore and is at first in a gelatinous condition which perhaps causes the surrounding pseudo-spores to become fastened to its surface. The spore wall increases rapidly in thickness (FIGS. 15, 16, PLATE 4) and hardens into a dense opaque substance to which the sterile cells are firmly attached.

Soon after the fusion of the two nuclei in the central cell, the protoplasts of the young pseudo-spores stain as if they are degenerating. Their nuclei become smaller and smaller (FIG. 13) and finally disappear (FIG. 14, PLATE 4). Their cytoplasm

becomes less and less dense and also finally disappears like the nuclei, leaving only the cell walls.

A mature spore ball of *Urocystis Cepulae* is composed of a fertile spore cell which is surrounded by a varying number of pseudo-spores. Spore balls taken from a mature pustule and mounted in distilled water measure from 19 to 20 microns in diameter, while the central fertile spores measure from 12 to 15 microns. Anderson (2) measuring fifty spores of *U. Cepulae* in lactophenol found the average diameter to be 16.15 microns. In fact, I find that the size of the spores of *U. Cepulae* approaches more nearly the size of the spores of *U. magica*, which Anderson gives as 22.19 microns.

In all the sections of developing and mature spore balls of *U. Cepulae* which I have examined, I have observed but one fertile spore cell in each spore ball. Great masses of these spore balls are formed in the mature pustule and are set free by the drying up and subsequent cracking of the host tissue.

#### DISCUSSION

My studies of *Urocystis Cepulae* show conclusively that the two nuclei that fuse in the young smut spore are not derived from a fusion of cells of the saprophytic mycelium, nor from a conjugation of conidia, since such processes do not occur in this species. The binucleate cells originate in the parasitic mycelium in the stages preceding the differentiation of the sorus primordium. Just how the binucleate condition arises has not been determined. The haploid stage extends over a considerable portion of the life cycle of the organism including the whole of the saprophytic stage by which the fungus maintains itself in the soil probably for years.

The developmental history of *U. Cepulae* is quite different from that which has been described for certain apparently closely related species, viz., *U. Anemones* and *U. Violae*. According to Kniep (26) in 1921, the haploid stage of *U. Anemones* consists of but four cells. The diploid stage begins with the conjugation of the three or four promycelial branches. Rawitscher (41) in 1922 gives a similar description for *U. Violae*, except that in this species seven or eight promycelial branches (conidia) are produced.

In its haploid stage *Urocystis Cepulae* more nearly resembles

*Doassansia Sagittariae*, as described by Rawitscher (41) in 1922, but in *D. Sagittariae* uninucleate conidia are produced, though, as is the case in *Ustilago Maydis*, they do not conjugate. Rawitscher further states that the binucleate condition originates a little before spore formation. It is his opinion that the binucleate condition is brought about by a dissolution of the cross-wall between two cells (p. 292), but he does not support this contention with figures. Rawitscher allies *D. Sagittariae* with *Ustilago Maydis* in its developmental history.

In my opinion, *Urocystis Cepulae* cannot be allied with *Ustilago Maydis* in its life cycle, since in the former the binucleate condition arises in the vegetative hyphae soon after infection, and not at time of spore formation, as described for *U. Maydis*. In this connection it is interesting to note that the phenomenon of the gelatinization of the cell septum (or the cell walls) of two adjacent uninucleate cells, by which the binucleate condition is initiated at time of spore formation, as maintained by Rawitscher (39) for *Ustilago Maydis*, has never been described for any other member of the Ustilagineae. Since this is a matter of such fundamental importance, verification of the observation for this or another species is desirable.

De Bary (17) states that in *Ustilago hypodytes* the membranes of the hyphae swell into a gelatinous substance so that each individual spore is soon surrounded by a broad hyaline gelatinous sheath. The definitive membrane of the spore is then formed on its outer surface inside the gelatinous envelope, which soon disappears as the spore matures (p. 175). De Bary's description of the gelatinization of the hyphal membrane is concerned with the separation of spore elements and the subsequent development of the spore wall, and not with cell fusion as maintained by Rawitscher for *Ustilago Maydis*.

In the light of the remarkable results reported by Sartoris (42) in 1924, one is inclined to wonder whether the binucleate structures which originate as the result of conjugation of conidia obtained on artificial media are viable and able to infect the host. Sartoris has shown convincingly that the secondary spores formed from the fused conidia of *Tilletia Tritici* are unable to infect the host. By growing *Ustilago Zeae* in a solution of maltose to which potassium dihydrogen phosphate and magnesium sulphate had been added, Sartoris obtained conjugation of



the conidia. Mycelium grown on an alkaline glycerin agar with special subsequent treatment formed smut spores. But "the spores are physiologically unlike the chlamydospores formed on the host plants . . . they are like them in appearance but these chlamydospores of *Ustilago Zeae* do not form a typical promycelium" (p. 636).

Since the conidia of *Ustilago Maydis* will not conjugate in ordinary media (dung-decoction) according to Brefeld (9) Rawitscher (39) and others, the conjugation of the conidia on 'special media' is significant. Obviously Sartoris (42) believes that his results indicate that the conjugation of the conidia of *U. Maydis* is induced when a sufficient amount of nutrient material is not available for the fungus. This coincides with statements of Brefeld (9) Dangeard (11) Harper (23) and Lutman (29). Sartoris also agrees with Lutman that the conjugation of the conidia represents an old form of sexuality which has ceased to play an essential rôle in the life cycle of the smuts (42, p. 638). According to Bauch (5) in 1923, in *Ustilago longissima* the binucleate cells conjugate freely with each other—a condition one would not expect of binucleate cells.

Thus the question arises: are the binucleate structures observed by Kniep (26) in *Urocystis Anemones* and by Rawitscher (41) in *U. Violae* viable and able to infect the host, or are they merely responses of the fungus to abnormal conditions? On this point the papers of Kniep and Rawitscher do not make any definite contribution. Without making any infection experiments, they assume that the binucleate structures (binucleate conidia) which originate by the conjugation of the mycelial branches of these species, will infect the host and thereby initiate the binucleate condition in the parasitic mycelium. Kniep and Rawitscher evidently based their conclusions as to the origin of the two nuclei that fuse in the young spore in *U. Anemones* and *U. Violae* on the following data: In *Ustilago Tragopogonis*, Rawitscher (39) in 1912, observed that the mycelium infecting young buds of *Tragopogon pratensis* is intercellular and is composed of binucleate cells. The binucleate condition of the mycelial cells and thus the young spore cells was considered by him to be brought about by the conjugation of conidia. Since, however, he did not include infection experiments in his work, one cannot be sure that this is the case. In 1922 Rawitscher

(41) extended his observations to *Tilletia Tritici* and *Cintractia Montagnei* with similar interpretations.

Rawitscher's contention that the conjugation of the conidia initiates the binucleate condition received substantial support by the further work of Kniep. In 1919 Kniep (25) produced evidence of a physiological nature in support of the belief in the sexual nature of the conidia that conjugate, by presenting data indicating the differentiation of the so-called plus and minus strains in the anther smut. He reports that in the anther smut two kinds of conidia arise from the promycelium. They are externally alike but physiologically different. Conjugation takes place only when the two different kinds of conidia are brought together. Conidia and cultures arising from a single conidium do not conjugate. Therefore, there exists between certain conidia a difference which he terms 'physiological sex differentiation.' In support of these statements Kniep isolated a great number of 'single conidial cultures' of the anther smut from spores obtained from various host plants, and succeeded in bringing about conjugation in the majority of cultures by combining the conidia with suitable mates. These observations of Kniep, together with his conclusion that *Ustilago violacea* is composed of biological forms, have been confirmed and extended by Bauch (4, 5, 6) and by Zillig (53). The work of these investigators seems to establish the sexual nature of conidial fusions, but it is evident that the nuclear history of a larger number of species must be worked out before full agreement as to the nuclear phenomena in the reproduction of the Ustilagineae can be expected.

The results of my study of *Urocystis Cepulae* conclusively show that the uninucleate saprophytic mycelial cells taken from cultures infect the host tissue and produce uninucleate parasitic mycelium in the host tissue, which, in turn, soon gives rise to binucleate mycelial cells. As stated above, all the cells of the young sorus of *Urocystis Cepulae* are binucleate, and these binucleate cells give origin to the spore ball primordia—the fertile spore itself taking its origin from one of the binucleate cells.

#### SUMMARY

1. Onion seedlings are infected by *Urocystis Cepulae* as they make their way through the soil. Infection occurs only through

the cotyledons. Within three to four weeks mature pustules appear on the cotyledons of the seedlings.

2. Mature spores from unopened sori were germinated in onion decoction or on onion agar. In the course of three to seven days the spores begin to germinate. All the spores do not germinate at once.

3. Germination begins by the protrusion of a hemispherical promycelium which buds out eight or a less number of hyphae of indeterminate growth. By continued growth and branching of these primary hyphae, a web of mycelium is produced about the spore which is visible to the eye in about ten days. Conidia are not produced.

4. The smut fungus was isolated and grown on various media as follows: onion agar, sterile potato, onion, bean, and carrot.

5. During spore germination the fusion nucleus divides within the spore. The nuclei migrate through the short promycelium to the promycelial branches. One nucleus passes into each promycelial branch, which segments ultimately into uninucleate cells. Conjugation between promycelial branches does not occur.

6. The saprophytic mycelium is uninucleate from the beginning and remains so throughout its existence.

7. Each mycelial cell of the saprophytic mycelium may function as an oidial spore. The oidial spores serve to propagate and disseminate the fungus in the soil.

8. The oidial spores are uninucleate and after germinating produce uninucleate mycelium, as do the resting spores.

9. Dessication does not kill the saprophytic mycelium nor the fungus in the soil.

10. Infection of the seedlings takes place directly from the mycelium, under experimental conditions, and probably in the field likewise.

11. The parasitic mycelium is intercellular throughout its existence.

12. The hyphal cells of the parasitic mycelium are at first uninucleate but become progressively binucleate as they approach the young sorus primordium, where all the cells of the sporogenous hyphae are binucleate.

13. The spore ball has its origin from a group of sporogenous branches, each of which consists of short binucleate cells.

14. The fertile spore takes its origin from a binucleate, centrally located cell of a sporogenous branch.

15. The two nuclei in the young spore soon fuse. The spore cell then increases rapidly in size, the pseudo-spores become appressed upon its surface.

16. The pseudo-spores have their origin from binucleate cells of the sporogenous branches, and serve as 'nurse' cells to the central fertile spore.

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#### Description of plates 1-4

The photographs were made with Carl Zeiss Protar and Planar lenses, having  $6\frac{1}{2}$  inches and 4 inches focal length respectively. Photographs were made natural size except where otherwise indicated: all have been slightly reduced in reproduction. All figures were drawn with the aid of the camera lucida and Zeiss 2 mm. apochromatic objective N. A. 1.30 and no. 12 compensating ocular.

#### Plate 1

FIGS. 1-6. Mycelium of *Urocystis Cepulae* growing on onion agar, representing various ages of the culture.

FIG. 1. Culture transferred from onion agar plate to onion agar, 11 days after germination. Photographed 2 days after transfer. A spherical mat of mycelium, white in appearance.

FIG. 2. Culture growing 3½ days after transfer. The mycelial threads can be observed to be growing radially from the oval cottony mass of mycelium.  $\times 5.5$ .

FIG. 3. The largest culture is 19 days old and developed from a single spore. The three upper and smaller mycelial mats represent secondary infection and are about 8 days old. Ridges on the surface of each.

FIG. 4. This represents a culture 16 days old, on onion agar. The characteristic wrinkles on the surface are apparent. A delicate fringe surrounds the periphery.  $\times 2$ .

FIG. 5. Culture transferred from culture on bean to onion agar. Fourth transfer from the original culture. Culture 30 days old.  $\times 2$ .

FIG. 6. Culture growing on onion agar, 69 days old. The characteristic ridges running radially. Along the lower edge is a fringe of growing hyphae that show zonal growth.

FIG. 7. *U. Cepulae* growing on sterile potato, 7 days' growth. The mycelium is snow-white and tends to accumulate in nodular masses.  $\times 5$ .

FIG. 8. Culture on potato, 51 days old. Because of certain conditions in the potato, the mycelial growth is being slowly destroyed. The mycelial mat at this stage is thin and the crests of the reduced ridges have broken open.

FIG. 9. Culture on sterilized onion, 68 days old. Growth at first was exceedingly slow. Finally a mat of mycelium appears with the characteristic ridges and wrinkles.

FIG. 10. Culture on a green but sterile onion, 47 days old. The mycelial mat covers a portion of the onion. Surface shows wrinkles, the crests of which are white. Spore formation does not occur. Second transfer.

## Plate 2

FIG. 1. *Urocystis cepulae* growing on sterilized bean pod, 7 days old. The mycelium piles up in layers to a depth much greater than on any other medium used. The whole mass appears white, due to the loose superficial hyphae which enclose air in the meshes of the mycelium.

FIG. 2. Culture on sterilized bean pod, 51 days old. The ridges on the surface of the mycelial mat are high and thick. The growth is rank. At the periphery of the culture is a fringe of actively growing mycelium which shows zonal growth.

FIG. 3. Culture on sterilized bean pod, 51 days old. Mycelium which was growing on onion agar was broken up into its cellular elements then sprayed on to the bean pod. Apparently wherever a mycelial cell (oidial spore) settled, it developed a weft of mycelium. Great numbers of the little mycelial colonies have fused.

FIG. 4. Culture on sterilized carrot, 25 days old. Due to slow growth on carrot, in the beginning, the mycelial mat is just commencing to show the characteristic wrinkled surface.

FIG. 5. Culture on sterilized carrot; later stage than FIG. 4, 47 days old. The characteristic wrinkling is evident. The wrinkles are not nearly so pronounced as on the onion agar or on the bean.

FIG. 6. Culture on sterilized carrot, 61 days old. The ridges have fused to such an extent that the whole surface is much smoother—just the opposite from that noted on the bean, FIG. 2.

FIG. 7. Mycelium growing on onion agar. Each mycelial mat developed from a mycelial cell (oidial spore). The surface appears wrinkled.

FIG. 8. Mycelium developed from spores taken from an unopened smut pustule, then placed on onion agar. Thus the mycelium in FIG. 7 is from oidial spores while that of FIG. 8 is from the resting spores.

### Plate 3

FIG. 1. Germinating spore of *U. Cepulae*. The subspherical promycelial vesicle is protruding from one side. The promycelium is budding from its surface the first primary hypha.  $\times 960$ .

FIG. 2. A later stage. Two primary hyphae are growing from the surface of the promycelium.  $\times 960$ .

FIG. 3. A spore with the promycelium. From the promycelium has developed one primary hypha, which consists of two cells, each uninucleate. The cell nearest the promycelium is budding off a branch.

FIG. 4. A later stage of germination than FIG. 3. Two primary hyphae are shown; one is branched and consists of uninucleate cells.  $\times 960$ .

FIG. 5. The germinating spore with hemispherical promycelium. Four primary hyphae are growing from the promycelium. The primary hyphae consist of uninucleate cells.  $\times 960$ .

FIGS. 6, 7, and 10. Cells of the saprophytic mycelium in different stages of activity.  $\times 1200$ . In older cultures some of the hyphae are composed of uninucleate cells of the appearance of FIG. 7, cytoplasm is dense and contains refractive granules. Other hyphae may consist of cells rounded at the ends and constricted in the middle, FIG. 6. Still others more desiccated, FIG. 10, which appear like a chain of oidial spores. Each cell is uninucleate.

FIG. 8. Hyphal cell germinating. The mycelial cells had been shaken apart and placed in onion decoction for 18 hours. The nuclei and cytoplasm have left the original cell and, in each case, migrated into the branch.  $\times 900$ .

FIG. 9. Represents the characteristic habit of the saprophytic hyphae, showing also the characteristic branching. Each cell of the hyphae is uninucleate. The hypha, as represented, is from the aerial portion of the culture, growing on onion agar.  $\times 1200$ .

FIG. 11. A germinating hyphal cell (oidial spore) in which the mother cell and the branch each contain a nucleus. A septum has not as yet been laid down between the nuclei.  $\times 1200$ .

FIG. 12. A portion of a mycelial thread which shows each uninucleate cell of the hyphae germinating in a characteristic fashion.  $\times 900$ .

FIG. 13. Mycelial cells recovered from the soil 10 hours after the soil had been infected with mycelium from the cultures. Each cell is beginning to germinate.  $\times 1200$ .

FIG. 14. Similar to FIG. 8 except that the nuclei have remained in each of the original mycelial cells (oidial spores). One cell is shown which is giving off a germ tube at each end.  $\times 1200$ .



## Plate 4

FIGS. 1-3. Binucleate cells of the parasitic mycelium that permeate the host tissue in the immediate vicinity of the young sorus.  $\times 1200$ .

FIGS. 4, 5. Uninucleate cells of the vegetative mycelium.  $\times 1200$ .

FIG. 6. Spore ball primordium measuring about 10 microns across; the hyphal cells one-half micron in diameter. The hyphal cells are binucleate. The large central cell is the primordium of the fertile spore.  $\times 1200$ .

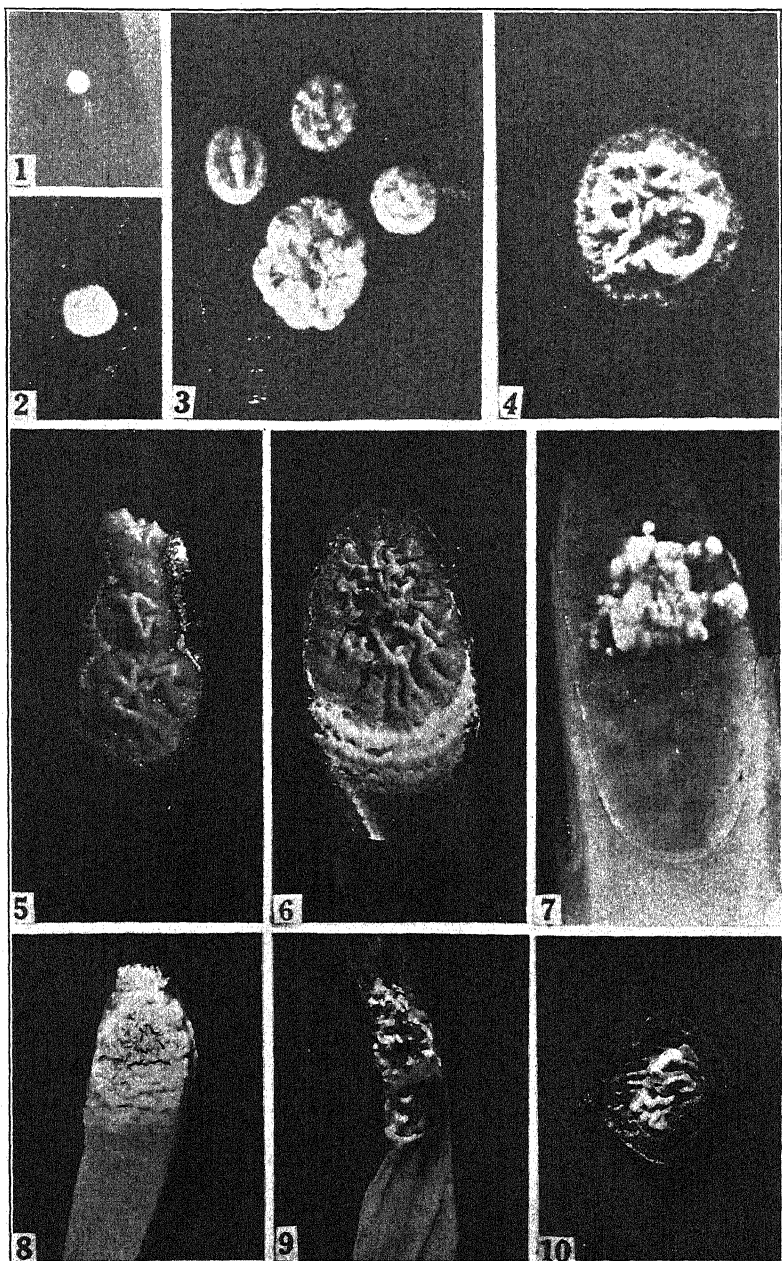
FIGS. 7-10. Spore ball primordium showing somewhat later stage. The binucleate central cell has increased in size. The cells of the surrounding sporogenous hyphae are each binucleate. FIG. 9. shows the young binucleate central cell increasing in size, causing the sporogenous branch from which it originated to be distorted. The two ends of the sporogenous branches are still attached to the young spore. The central cell measures  $2 \times 3$  microns. (FIGS. 7-9,  $\times 1200$ , FIG. 10,  $\times 900$ .)

FIG. 11. The two nuclei are in close proximity and apparently fusing. The surrounding hyphal cells are binucleate.

FIGS. 12, 13. The now uninucleate central cell has increased in size while the pseudo-spores appear uninucleate with degenerating cellular contents.  $\times 1200$ .

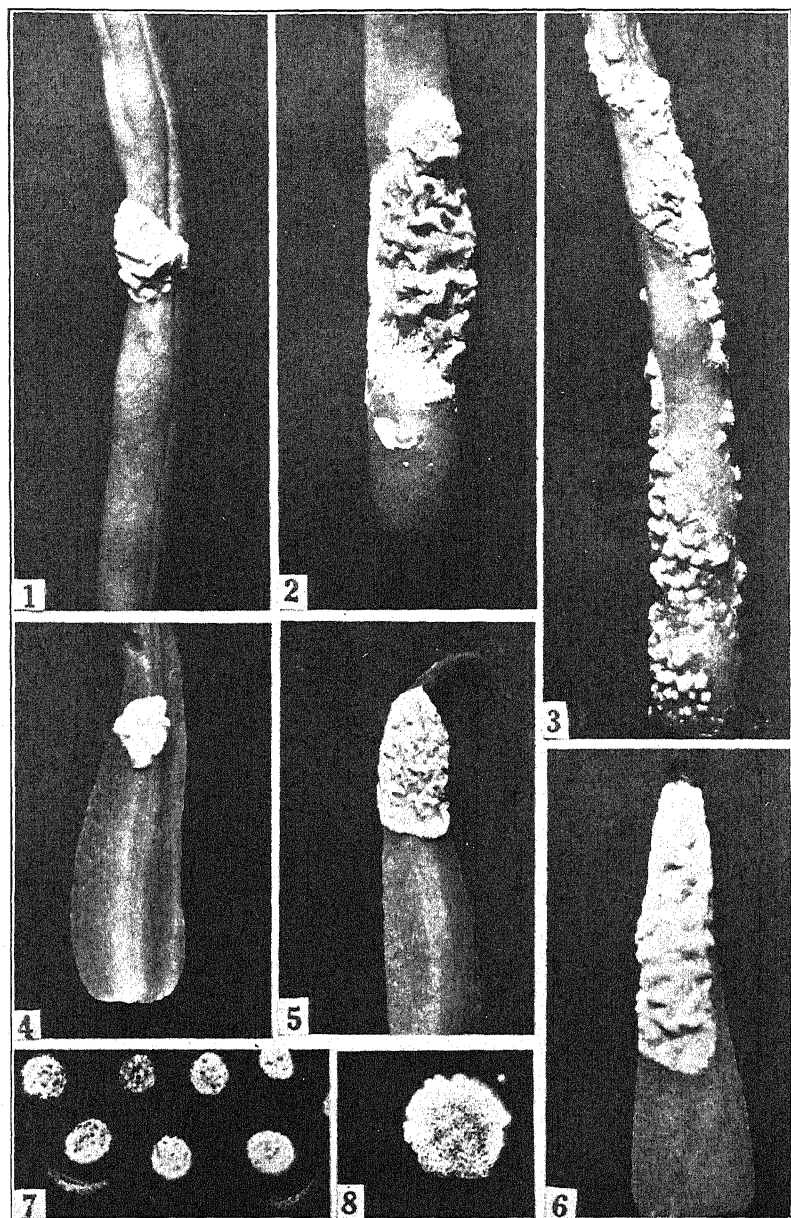
FIGS. 14-16. The central spore has increased to such proportions that it presses against the pseudo-spores which now adhere firmly to its surface. The pseudo-spores now contain no nuclei and very little cytoplasm.  $\times 1200$ .





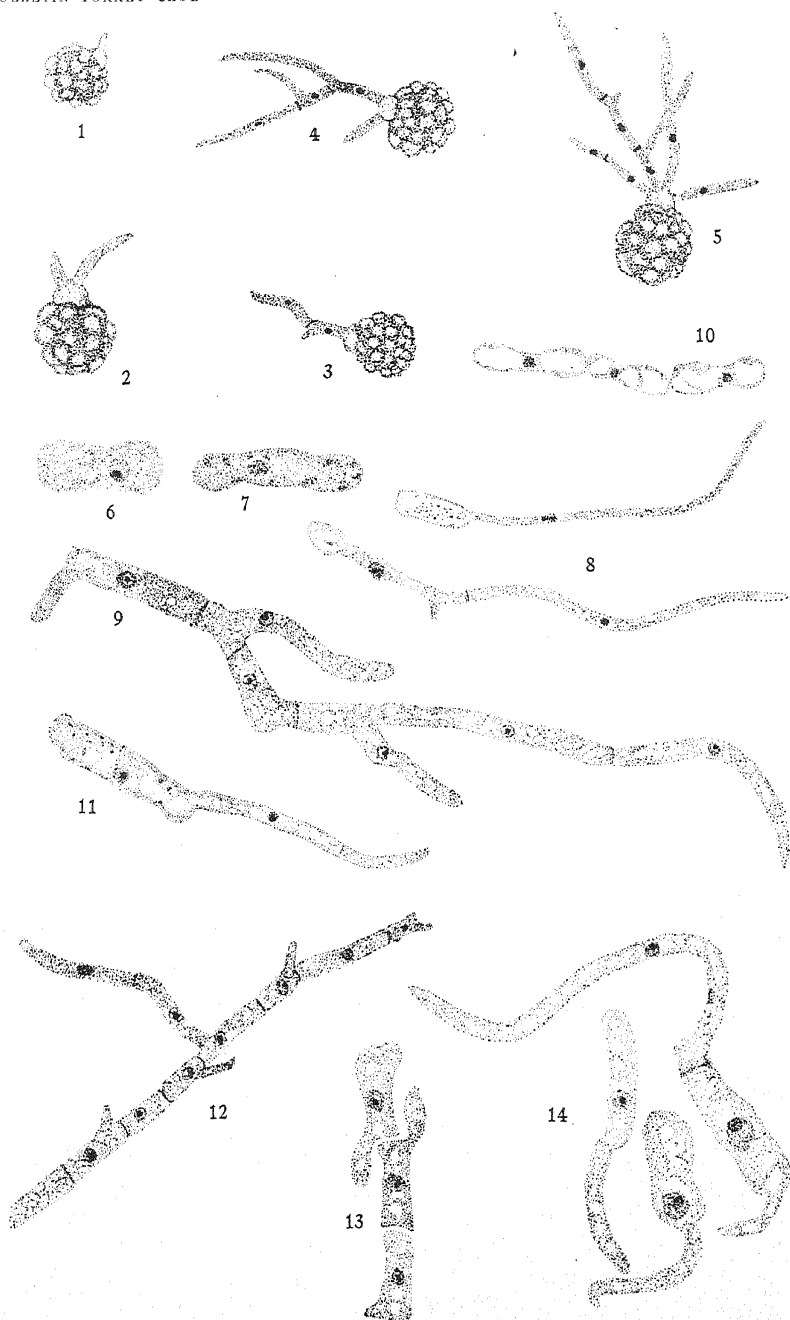
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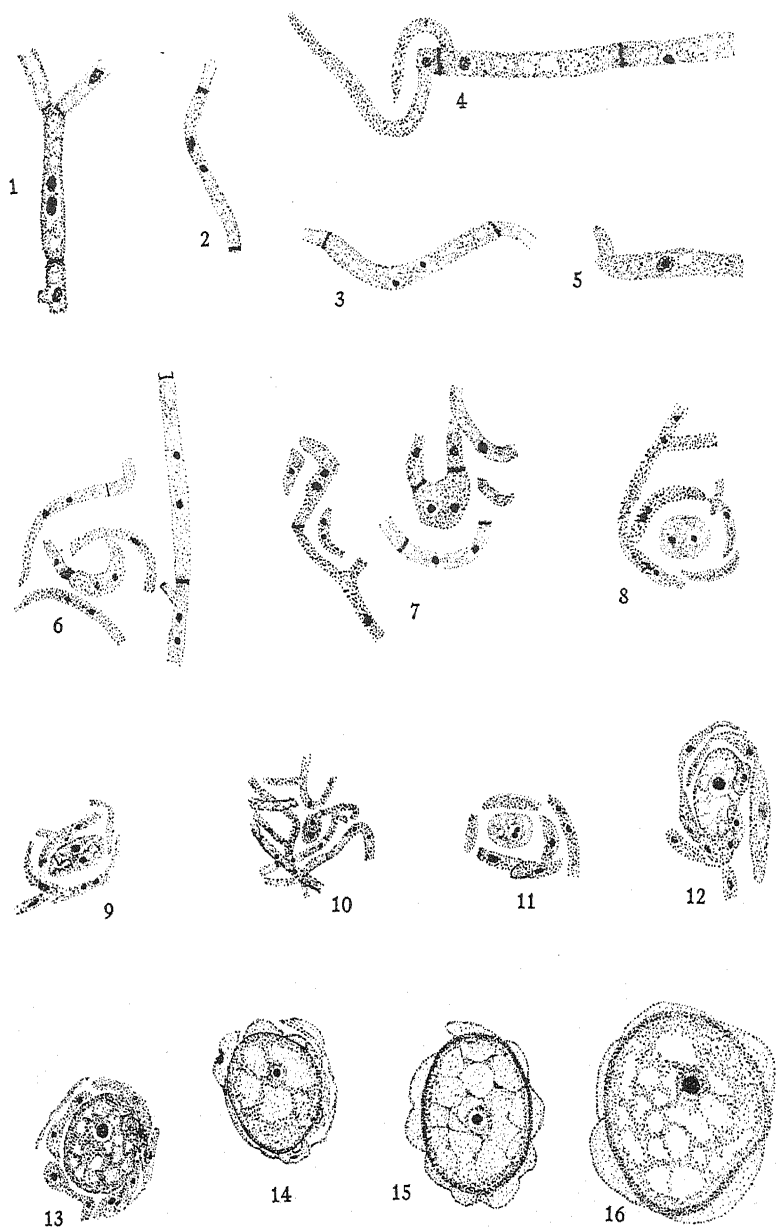




UROCYSTIS CEPULAE FROST







UROCYSTIS CEPULAE FROST



# INDEX TO AMERICAN BOTANICAL LITERATURE

1925

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BULLETIN  
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MARCH 1926

The desert of northern Baja California

FORREST SHREVE

(WITH TWO TEXT FIGURES)

The peninsula of Lower California is one of the most thinly settled and poorly explored portions of North America, outside the Arctic regions. The aridity of the climate and the absence of generous sources of water has retarded the development of even such small areas as might have agricultural value. With an area nearly half as great as that of the State of California, the peninsula has a population of only 42,000. With the scarcity of settlements in the interior and the absence of roads, the exploration of Lower California has been chiefly along the coast. An expedition led by E. A. Goldman and E. W. Nelson in 1905-06, under the auspices of the Biological Survey, covered the peninsula more thoroughly than earlier workers had been able to do, and resulted in the most important publications that have appeared on the distributional and ecological aspects of the vegetation.

The plant and animal life of Lower California bears many resemblances to the biota of Southern California and of the mainland of Sonora and Sinaloa. In the central and southern part of the peninsula, however, there are many endemic forms, indicating the long duration of practically insular conditions of isolation. In addition to many striking species of palms, yuccas, cacti and desert trees and shrubs, the flora of the peninsula contains a number of plants of unusual form and habit, calculated to give zest to the exploration of every mountain range and valley. There is the procumbent giant cactus, *Lemaireocereus eruca*, the large and richly branched *Fouquieria peninsularis*, as well as the closely related *Idria columnaris*, with stout, abruptly tapering stem, two branching arborescent species of *Nolina*, and several

[The BULLETIN for February (53: 59-128) was issued 27 February 1926].

trees in which the diameter of the greatly thickened trunk is out of all proportion to the height.

A broken series of mountain ranges runs through the entire length of Lower California, with several low passes connecting the outwash slopes of the Pacific Coast with the narrower slopes that face the Gulf of California. The highest elevation is Santa Catarina Peak, 10,135 feet in elevation, in the San Pedro Martir Mountains, 125 miles south of the International Boundary. Other ranges further south reach elevations of 5000 to 8000 feet. From the northern base of the San Jacinto Mountains, in Southern California, to the southern end of the San Pedro Martir range there is a series of elevations almost continuously above 5000 feet, and extending parallel to the Pacific Coast for 300 miles. In the lee of these mountains is one of the most arid parts of America north of the equator. Rain is confined to the winter and early spring months, and falls only when rain on the coast or in the mountains is accompanied by winds of considerable strength and duration.

In November, 1924, three members of the staff of the Desert Laboratory made a short expedition from the Salton Basin down the eastern coast of Lower California to a locality nearly opposite the southern end of the San Pedro Martir Mountains. A little-used road was followed as far as San Felipe Bay, from which place a short excursion to the south and interior was made on foot. The preceding summer had been an unusually dry one throughout the lower basin of the Colorado River, and the preceding winter had also been deficient in rainfall. The region was seen, therefore, in a condition such as to give an adequate conception of the conditions which must be met by the plants and animals of this extreme desert.

After crossing the International Boundary at Mexicali our route lay across the irrigated alluvial lands which share in the water of the Colorado River that is taken out by the Imperial Canal. The sole crop here is cotton, which produces a heavy yield from large perennating plants. On emerging from the irrigated lands the Cucupa Mountains lie on the right, and on the left the delta of the Colorado River. This part of the delta has a heavy stand of cottonwood (*Populus Macdougalii*), mesquite (*Prosopis glandulosa*), arrow weed (*Pluchea sericea*), and salt bush (*Atriplex spp.*). The narrow bajada of the mountains is

covered with a very open stand of *Covillea*, infrequent individuals of *Parkinsonia*, and with numerous plants of *Isocoma veneta*. The only other perennials are the two cacti *Opuntia echinocarpa* and *O. ramosissima*, and infrequent plants of *Asclepias subulata*.

The bajada of the Cucopa Mountains becomes very narrow on passing southward and finally vanishes at El Mayor, where Hardy's Colorado cuts the base of the mountains. From this locality the Hardy flows in a southeasterly direction, its flow

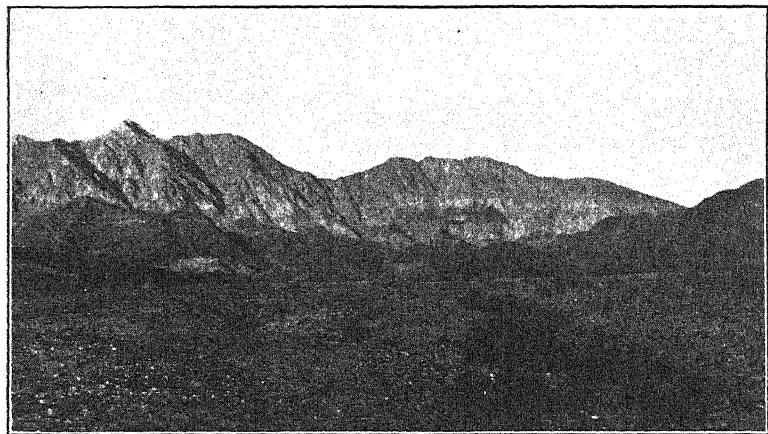


FIG. 1. Eastern slopes of the Cucopa Mountains at El Mayor, showing barren slopes and bajada with sparse stand of *Covillea*.

soon becoming subject to tidal influence. South of El Mayor is an extensive tidal flat which borders the Hardy and the head of the Gulf for a distance of over fifty miles, and has a width varying from a few miles at the north to over twenty miles at its center. This flat is covered with shallow water at times when the Colorado River is in flood concurrently with spring tides in the Gulf. At the time of our visit the surface had been dry for several months and was coarsely broken by conchoidal fractures. The landward edges of the flat have a very thin stand of small mesquites at the northern end, many of which have been killed by the continued saturation of the substratum which is so fatal to this tree. The principal part of the flat is wholly devoid of vegetation. At the southern end of the Cucopa

Mountains, and among the smaller volcanic ranges immediately south of them, the customary development of bajadas has been prevented by the removal of outwash material by tidal action or its distribution over the level surface of the mud flat.

The slopes of the Cucopas facing the east are more nearly devoid of vegetation than any hills or mountains that the writer has seen in the southwestern deserts, not excepting those in the central part of the Mojave Desert. On several of the lower slopes that were examined there were no perennials and it was impossible to find traces of plants that might have grown there during rainy periods. It is very likely, however, that the vigorous winds which visit this region may have removed all evidence of herbaceous ephemerals, as there is always enough sand in motion near the ground in a high wind to cut the small dry stems. Other slopes that were visited bore a few small individuals of the barrel cactus *Ferocactus acanthodes* (*Echinocactus cylindraceus*). Near the bases of other slopes were a few individuals of *Covillea tridentata* and *Franseria dumosa*. On one hill there persisted a few tufts of a grass, apparently *Heteropogon contortus*. It is doubtful if there are any places in the western hemisphere where the landscape is more completely devoid of plant life than it is throughout the 30 or 40 miles of our journey in which the tidal flat formed the foreground and the barren hills rose abruptly from its inner edge.

The tidal flat was traversed to within about fifteen miles of its southern end, when both the Cucopa Mountains and the Sierra Pinta had been left behind. South of the latter range the nearest mountains are from five to twenty miles inland, and their bajadas drain down to the inner edge of the tidal flat. Here the alluvial soil of the flat receives an admixture of sand and infrequent irrigation with fresh water, and there is consequently a narrow zone of halophytic vegetation, chiefly *Frankenia Palmeri*, with occasional groups of *Spirostachys occidentalis*.

The bajadas south of the Sierra Pinta are covered with a relatively heavy stand of perennial vegetation, there being approximately from 40 to 60 individuals to the acre. The few species seen here are all common in similar situations throughout western Arizona and Sonora, including *Prosopis glandulosa*, *Olneya tesota*, *Covillea tridentata*, *Parkinsonia Torreyana* (Cer-



*cidium*), and *Franseria dumosa*. The cacti are conspicuously infrequent, being represented only by widely separated individuals of *Ferocactus acanthodes* and *Opuntia echinocarpa*. The surface of the bajada is like that in adjacent regions with very low rainfall, with a close network of small shallow drainageways and with no large ones.

South of the region just described it is necessary to traverse nearly twenty miles of light sand, which stretches from the mountains to the Gulf at this place. In this area, *Parkinsonia* and *Olneya* are absent and *Prosopis* is relatively infrequent, the dominant perennials being *Covillea*, *Acacia paucispina* and *Franseria dumosa*. All of the larger bushes in this wind-swept area have accumulated hummocks of sand, and groups of small dunes from six to twelve feet in height are common, always fairly well stabilized by an open cover of *Acacia* or *Covillea*.

The remainder of the journey to San Felipe Bay was across the bajadas of hills which rise from five to fifteen miles from the coast of the Gulf. On some of them there is a hard surface covered with desert pavement of small stones, on others a great deal of wind-blown sand, and on the greater part of them a soil which appears to be a loam with considerable coarse sand or fine gravel, which, however, is of very much finer texture than the surface material would indicate. Low and open stands of *Covillea*, with a few individuals of *Franseria* and *Acacia*, form the prevailing vegetation of this region. In many spots the number of plant individuals is less than twenty per acre. Under low bushes it is possible to find loose wind-blown fragments of herbaceous plants, which had been blowing about for eight months, or some of them possibly for twenty months. In this material it was possible to recognize species of *Cryptanthus*, *Amsinckia*, *Chorizanthe*, *Gilia* and *Plantago*.

Although the bajadas in general are without streamways, other than very shallow ones less than two or three feet in width, there are some places in which the configuration of the surface concentrates the very infrequent run-off into streamways from 15 to 75 feet in width. Along these streamways and in them are the heaviest stands of trees, with the largest individuals. *Prosopis*, *Olneya*, *Parkinsonia Torreyana* and *Parosela spinosa* here reach heights of 20 to 25 feet. The latter is one of the most striking trees of the Colorado and Gulf deserts. It is

without leaves in the dry seasons, is very richly branched, and its stems and twigs have a bluish gray color which almost masks the green and gives to the tree its popular name of "smoke tree." When it is in bloom, covered with a profusion of magenta flowers, it is one of the most unforgettable sights of the desert.

On reaching San Felipe Bay, camp was made near the well of slightly brackish water which affords the only supply in this region. The well is at the northern end of a narrow valley, with extremely fine alluvial soil, lying just back of the crest of the beach. The valley is covered with a sod of *Distichlis spicata*,

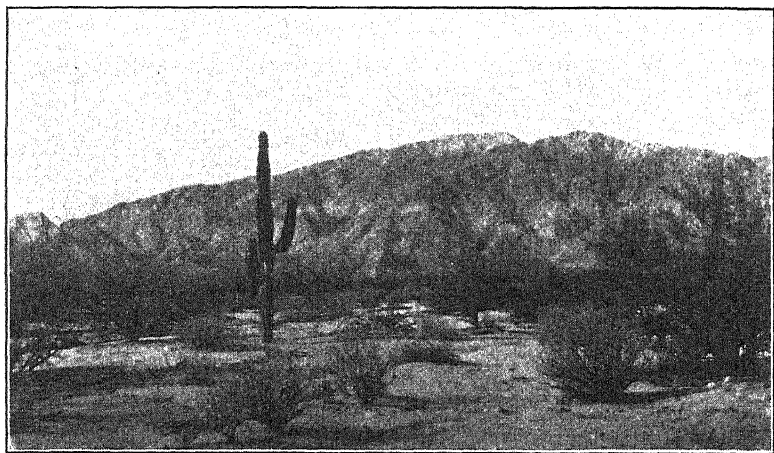


FIG. 2. Upper bajada west of San Felipe Bay. The cactus is *Pachycereus Pringlei*; to its left, *Elaphrium microphyllum*; to its right, *Elaphrium Macdougalii* and *Parkinsonia microphylla*; in the foreground, *Encelia farinosa*.

and has a few mesquite trees, which appear to be *Prosopis velutina*, rather than *P. glandulosa*, which is the common tree of the region. The northern limit of San Felipe Bay is formed by a short range of hills, and the southern boundary by lower hills, from which an extensive area of dunes and sandy soil extends inland to the mountains, marking the southern limit of progress with wheeled vehicles.

Directly west of San Felipe Bay the nearest mountains rise at a distance of about eight miles. The bajadas were explored on foot, and were found to bear a progressively heavier stand of vegetation as the mountains were approached. The commonest

forms are *Covillea*, *Acacia*, *Parkinsonia*, *Fouquieria*, *Encelia* and *Franseria*. Less frequent are *Olneya*, *Simmondsia* and a large *Krameria*.

*Fouquieria* here reaches the greatest size that has been observed anywhere in its range, being from 18 to 26 feet in height, sometimes with over one hundred branches, and commonly much more richly branched than at the northern edge of its range. A frequent tree here and for some twenty miles to the north is *Elaphrium microphyllum*, growing with the less frequent *E. Macdougalii*. This is the copal tree, also found in Sonora, the northernmost of the trees with greatly thickened and rapidly tapering trunk. The only other perennials not found in California are the columnar cereus, *Pachycereus Pringlei*, which is very infrequent here, and the *mochi*, *Lophocereus Schottii*, which is also infrequent. The only other cacti noted on the upper bajadas were *Opuntia Bigelovii*, *O. fulgida*, *O. ramosissima*, and *Ferocactus acanthodes*. All of these species are very infrequent. The lower slopes of the mountains west and southwest of San Felipe were very similar in their barrenness to those that have been described for the Cucopa Mountains.

The upper bajadas that have just been described were more thickly covered with plants than any of the other localities observed in the relatively short distance over which the coast was examined. The vegetation of these thickest stands is still very open and also very much poorer in species than similar topographic sites in Arizona or the interior of Sonora. The absence of the sahuaro, *Carnegiea gigantea*, and the presence of the copal tree give the landscape a very different aspect here. The slopes of the mountains form an even stronger contrast to those of regions with as much as ten inches of annual rainfall.

The vegetative activities of desert plants differ chiefly from those of moister regions in being intermittent. After heavy rains the desert plants grow, form leaves, bloom, and set seed with remarkable rapidity, and such seeds as are in the ground germinate and grow with equal rapidity. During a large part of the year, however, these plants are inactive and merely existing, with transpiration and respiration cut down to the lowest minimum. The different sections of the southwestern desert differ chiefly in the frequency and length of the periods in which the plants are quiescent. It is obvious that life and

growth, reproduction and migratory movements are more difficult in proportion as the quiescent periods are longer. The region between Imperial Valley and San Felipe Bay will always be one of great interest because its vegetation is the resultant of the highly intermittent activity of its plants through many centuries of deficient and irregular rainfall.

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## Toxicity and antagonism in salt solutions as indicated by growth of wheat roots<sup>1</sup>

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(WITH FIVE TEXT FIGURES)

A simple method of studying root elongation in very young seedlings has been described in a previous paper (Trelease and Trelease, 1925). It was found that reliable solution-culture results may be secured if attention is confined to the first few days of seed germination and that by means of such tests physiological balance may be demonstrated in a quantitative manner. It is the purpose of this paper to present results which indicate that the same method may also be used in studying toxicity of single-salt solutions as well as antagonistic effects in mixed solutions.

It has long been known that a culture solution containing a single salt is usually toxic to plants, and that the toxicity of such a solution may in many cases be diminished by the addition of another salt, which would also be toxic in simple solution. This phenomenon of antagonism is of such fundamental importance in physiology that it has, of course, received much attention in the literature. Reference may be made here to the valuable discussions of the literature given by Stiles and Jörgensen (1914) and by Stiles (1923) as well as to a summary of Osterhout's important contributions to our knowledge of antagonism for plant tissues (Osterhout, 1922).

Much less is known of the causes of antagonism than of its effects. Osterhout (1922), who has developed an elaborate hypothesis to explain antagonism, supposes that the antagonistic salts combine with some constituent of the protoplasm (at the external surface of the cell or at internal surfaces) to form new compounds. As a result of the antagonistic action, the salts in a balanced solution penetrate living cells much more slowly than do salts in an unbalanced solution, and the slow penetration of antagonistic substances has no unfavorable influence on the life processes so long as the substances within the cell remain properly balanced.

<sup>1</sup> Contributions from the Department of Botany of Columbia University, no. 342.

It is probable that the causes of antagonism must be sought by determining the laws governing the absorption of salts, or ions, and by determining the nature and controlling conditions of the chemical reactions produced in the cell walls and at the surface and in the interior of the protoplasm of the cells. Nevertheless, a complete explanation of the phenomena will require much more information than we now possess regarding the effects of antagonism on growth and the processes which accompany it.

Many of the results reported in the literature of salt antagonism have been based on experiments with a very small number of plants. Although such tests illustrate antagonistic effects qualitatively, the quantitative significance of the data has in many cases been obscured, probably by the high degree of variability of the plants as well as by the complexity of the other influential conditions.

The present work was undertaken with the aim of determining whether root elongation of very young seedlings might be used for studies of antagonistic effects. For this purpose, a study was made of the rate of elongation of wheat roots immersed in solutions containing pairs of the salts, potassium nitrate ( $\text{KNO}_3$ ), calcium nitrate ( $\text{Ca}(\text{NO}_3)_2$ ), and magnesium nitrate ( $\text{Mg}(\text{NO}_3)_2$ ), and in simple solutions of each of these salts. Every two-salt solution had a total concentration of 0.06 gram-molecule per liter, but in the various solutions of each series the volume-molecular proportions of the constituent salts were varied in a systematic manner. Since the various two-salt solutions were alike in molecular concentration, they differed from one another in osmotic concentration; values for the latter ranged from about 3 to about 4 atmospheres. The tests with single-salt solutions included a rather wide range of concentrations. A comparison was made of growth rates in the experimental solutions and in a standard three-salt solution, and the retardation of growth in the experimental solutions was used as the criterion of their toxicity.

These experiments were begun at the University of Louisville, and continued at Columbia University. It is a pleasure to acknowledge indebtedness to Professor Burton E. Livingston, of the Johns Hopkins University, for valuable suggestions in the preparation of this paper.

## METHODS AND RESULTS

The culture methods were essentially the same as described in a previous paper (Trelease and Trelease, 1925). Seeds of a pure-line spring wheat (Marquis, Saskatchewan, no. 70, supplied by the University of Saskatchewan, through the kindness of Professor Manley Champlin) were soaked for three hours in tap water and then sprouted on wet blotting paper in a moist chamber. Each culture vessel consisted of a glass tumbler (capacity, 275 cc.) having a piece of paraffined bobbinet stretched over the top and fastened by a ligature of paraffined linen thread. The tumbler was placed in a 600-cc. beaker (Griffin low form), and both the tumbler and the space around it were filled with solution, the level of the latter being even with the top of the tumbler.

When the first root of each seedling was about 6 mm. long, the seedlings were placed upon the netting at the surface of the solution, so that every root dipped into the solution while the seed was exposed to the air. Duplicate cultures, each of 25 seedlings, were grown. The cultures were kept in darkness, and during the first two days each was covered with a watch glass. The duration of the test was the time required for the roots in a standard or control solution to elongate about 84 mm. (from 6 mm. to 90). This standard solution contained 0.02 M  $\text{KH}_2\text{PO}_4$ , 0.02 M  $\text{Ca}(\text{NO}_3)_2$ , and 0.02 M  $\text{MgSO}_4$ , and allowed relatively rapid growth of roots. At the end of the test the length of the longest root of each seedling was recorded. For each culture, the mean initial root length (about 6 mm.) was subtracted from the mean final root length, and the value for elongation thus obtained was expressed as a percentage of the average elongation for the standard solution. Thus, each value for elongation represents an average growth rate during a time-period defined by the average growth rate in a standard solution. The results of the various tests are summarized in TABLES 1-4, and they are shown graphically in FIGS. 1-5.

Attention should be called to the fact that these data do not show how the growth rate may have changed for successive time intervals within the experiment period. Nor do they permit comparisons to be made between the time periods required for the roots in the various solutions to make equal amounts of growth (Osterhout, 1922). Further studies are needed if information is to be secured concerning these features of the growth phenomena.

TABLE I  
*Growth of wheat roots in salt solutions\**

CONCENTRATION		KNO <sub>3</sub>			Ca(NO <sub>3</sub> ) <sub>2</sub>				Mg(NO <sub>3</sub> ) <sub>2</sub>	
Percentage of 0.06 M	M†	A	B	Ave.	A	B	C	D	Ave.	Ave.
0	0	—	—	82‡	—	—	—	—	—	82‡
1	.0006	—	—	—	—	—	—	—	—	20
2	.0012	—	—	—	52	56	—	—	—	15
4	.0024	—	—	—	38	44	—	—	—	11
10	.0060	80	73	77	28	30	33	31	31	8
20	.0120	54	59	57	29	30	31	29	30	9
30	.0180	34	36	35	28	30	35	31	31	6
40	.0240	30	25	28	37	37	40	34	37	4
50	.0300	23	22	23	38	41	38	41	40	5
60	.0360	21	19	20	—	—	38	41	40	4
70	.0420	15	16	16	39	43	39	42	41	3
80	.0480	14	13	14	—	—	41	43	42	3
90	.0540	14	11	13	37	38	40	40	39	3
100	.0600	7	10	9	36	37	35	37	36	2
200	.1200	1	1	1	19	22	—	—	21	1
300	.1800	0	0	0	0	1	—	—	—	—
400	.2400	0	0	0	0	0	—	—	—	—
600	.3600	—	—	—	0	0	—	—	0	—

\* Duration of experiment the time required for roots in standard solution to elongate about 84 mm. (from 6 mm. to 90 mm.). Root growth expressed as percentage of elongation for standard solution containing 0.02 M KH<sub>2</sub>PO<sub>4</sub>, 0.02 M Ca(NO<sub>3</sub>)<sub>2</sub>, 0.02 M MgSO<sub>4</sub>.

† M denotes the volume-molecular concentration, or the number of gram molecules contained in each liter of solution.

‡ The mean (82) for distilled water is based on 14 cultures, as follows: 78, 79, 86, 82, 82, 79, 86, 84, 85, 80, 83, 82, 78, 80.



In the various series the temperature ranged from about 18° to 23° C., the mean temperature usually being about 19° C. At this temperature the roots in the standard solution required about 90 hours to elongate 84 mm.

TABLE 2

*Growth of wheat roots in solutions of potassium nitrate and calcium nitrate, the total concentration of each solution being 0.06 M\**

VOLUME-MOLECULAR PROPORTIONS†		ROOT GROWTH			GROWTH IN TWO-SALT SOLUTION MINUS GROWTH IN KNO <sub>3</sub> ‡	GROWTH IN TWO-SALT SOLUTION MINUS GROWTH IN Ca(NO <sub>3</sub> ) <sub>2</sub> ‡
KNO <sub>3</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	A	B	Ave.		
0	100	—	—	36	—	—
10	90	71	69	70	- 7	+31
20	80	76	67	72	+15	+30
30	70	79	76	78	+43	+37
40	60	83	82	83	+55	+43
50	50	82	84	83	+60	+43
60	40	90	85	88	+68	+51
70	30	87	80	84	+68	+53
80	20	83	86	85	+71	+55
90	10	80	80	80	+67	+49
100	0	—	—	9	—	—

\* Duration of experiment the time required for roots in standard solution to elongate about 84 mm. (from 6 mm. to 90 mm.). Root growth expressed as percentage of elongation for standard solution containing 0.02 M KH<sub>2</sub>PO<sub>4</sub>, 0.02 M Ca(NO<sub>3</sub>)<sub>2</sub>, 0.02 M MgSO<sub>4</sub>.

† Expressed as percentages of 0.06 M.

‡ Concentration the same as that in which the salt exists in the mixed solution.

#### DISCUSSION OF RESULTS

*Toxicity of single-salt solutions.* The graphs of FIG. 1 show the relations between growth rates and volume-molecular concentrations of single-salt solutions. It will be observed that every single-salt solution allows less rapid growth than occurs in distilled water or in the standard solution (the latter being a physiologically fairly well balanced solution). The present results thus agree with what generally has been found true for single-salt solutions. For successively higher concentrations, the growth rates decrease rapidly at first and then more and more slowly. While the curvature of the graphs for KNO<sub>3</sub> and for

TABLE 3

*Growth of wheat roots in solutions of calcium nitrate and magnesium nitrate, the total concentration of each solution being 0.06 M\**

VOLUME-MOLECULAR PROPORTIONS†		ROOT GROWTH			GROWTH IN TWO-SALT SOLUTIONS MINUS GROWTH IN $\text{Ca}(\text{NO}_3)_2$ ‡	GROWTH IN TWO-SALT SOLUTION MINUS GROWTH IN $\text{Mg}(\text{NO}_3)_2$ ‡
$\text{Ca}(\text{NO}_3)_2$	$\text{Mg}(\text{NO}_3)_2$	A	B	Ave.		
0	100	—	—	1	—	—
10	90	33	33	33	+ 2	+31
20	80	62	61	62	+32	+59
30	70	69	68	69	+38	+66
40	60	70	71	71	+34	+67
50	50	70	69	70	+30	+65
60	40	68	65	67	+27	+63
70	30	67	63	65	+24	+59
80	20	65	63	64	+22	+56
90	10	57	56	57	+18	+49
100	0	—	—	36	—	—

\* Duration of experiment the time required for roots in standard solution to elongate about 84 mm. (from 6 mm. to 90 mm.). Root growth expressed as percentage of elongation for standard solution containing 0.02 M  $\text{KH}_2\text{PO}_4$ , 0.02 M  $\text{Ca}(\text{NO}_3)_2$ , and 0.02 M  $\text{MgSO}_4$ .

† Expressed as percentages of 0.06 M.

‡ Concentration the same as that in which the salt exists in the mixed solution.

$\text{Mg}(\text{NO}_3)_2$  is of the common type, that of the graph for  $\text{Ca}(\text{NO}_3)_2$  is exceptional, since this graph at first falls, then rises, and finally falls again.

The relative growth-retarding effects of the three salts are in general agreement with most of the published data. Every tested concentration of  $\text{Mg}(\text{NO}_3)_2$  retarded growth much more than did the same concentration of  $\text{KNO}_3$  or of  $\text{Ca}(\text{NO}_3)_2$ . Even the lowest tested concentration of  $\text{Mg}(\text{NO}_3)_2$ , 0.0006 M, had a marked retarding effect. It is interesting to note that at low concentrations  $\text{KNO}_3$  retarded growth less than  $\text{Ca}(\text{NO}_3)_2$ , while at high concentrations the reverse was true.

Stimulating effects on growth, such as have been observed by Jensen (1907), Brenchley (1914), and other workers, are not evident from the present results. The growth of the roots in distilled water was more rapid than in any of the single-salt solutions.

It will be seen from FIG. 1 that the concentrations that

prevent any growth during the experiment period are about 0.060 M for  $\text{Mg}(\text{NO}_3)_2$ , 0.120 M for  $\text{KNO}_3$ , and 0.240 M for  $\text{Ca}(\text{NO}_3)_2$ . But it may not be possible to determine such concentrations with any high degree of accuracy, since each of the graphs tends to make a very small angle with the base line.

TABLE 4

*Growth of wheat roots in solutions of potassium nitrate and magnesium nitrate, the total concentration of each solution being 0.06 M\**

VOLUME-MOLECULAR PROPORTIONS†		ROOT GROWTH			GROWTH IN TWO-SALT SOLUTION MINUS GROWTH IN $\text{KNO}_3$ ‡	GROWTH IN TWO-SALT SOLUTION MINUS GROWTH IN $\text{Mg}(\text{NO}_3)_2$ ‡
$\text{KNO}_3$	$\text{Mg}(\text{NO}_3)_2$	A	B	Ave.		
0	100	—	—	1	—	—
10	90	5	4	5	-72	+3
20	80	5	6	6	-51	+3
30	70	9	9	9	-26	+6
40	60	9	8	9	-19	+5
50	50	11	13	12	-11	+7
60	40	14	14	14	-6	+10
70	30	17	19	18	+2	+12
80	20	26	29	28	+14	+20
90	10	32	36	34	+21	+26
100	0	—	—	9	—	—

\* Duration of experiment the time required for roots in standard solution to elongate about 84 mm. (from 6 mm. to 90 mm.). Root growth expressed as percentage of elongation for standard solution containing 0.02 M  $\text{KH}_2\text{PO}_4$ , 0.02 M  $\text{Ca}(\text{NO}_3)_2$ , 0.02 M  $\text{MgSO}_4$ .

† Expressed as percentages of 0.06 M.

‡ Concentration the same as that in which the salt exists in the mixed solution.

The present results do not, of course, allow accurate inferences to be drawn regarding the limits of endurance of these wheat plants to the salts employed. Special studies of this phase of the problem have been made by Harter (1905) and Jensen (1907).

That the comparative growth rates in different salt solutions may depend upon the duration of the test has been emphasized by Magowan (1908), who pointed out that after six days wheat plants in  $\text{NaCl}$  and  $\text{KCl}$  solutions had made more growth than those in  $\text{CaCl}_2$  solutions, but after twenty-five days the plants in  $\text{CaCl}_2$  solutions were in advance of the others. It is clear therefore that comparisons cannot be made between experi-

ments of different durations until special tests have been made of the relations of the time-period, or of the developmental stage of the plants, to the toxic action of salt solutions.

Certain rather striking differences in the appearance of the roots were noticed. Roots that had grown in concentrations of  $\text{Ca}(\text{NO}_3)_2$  lower than 0.03 M were covered with a heavy mat of long root hairs, this development of hairs being most conspicuous in the lowest concentrations and nearly absent in concentrations above 0.048 M. This action of calcium salts has been observed by a number of workers; Magowan (1908) refers to a similar

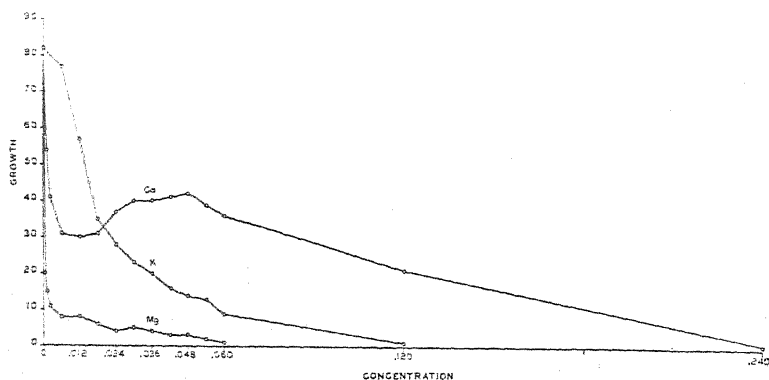


FIG. 1. Growth of wheat roots in single-salt solutions of nitrates of calcium (Ca), potassium (K), and magnesium (Mg).

Ordinates represent root growth, expressed as percentages of elongation for standard solution. Abscissas represent volume-molecular concentrations.

effect of  $\text{CaCl}_2$  solutions, favoring the development of root hairs. The roots appeared more slender in higher concentrations. In the highest concentrations (0.24 and 0.36 M) of  $\text{Ca}(\text{NO}_3)_2$ , the roots were white and the same in appearance as when placed in the solutions. Roots that had grown in the higher concentrations of  $\text{KNO}_3$  were translucent, swollen, soft and easily broken. The solution in which they had grown was opalescent. In the higher concentrations of  $\text{Mg}(\text{NO}_3)_2$ , five roots usually developed about equally, whereas in concentrated solutions of the other salts only one or three roots usually developed.

*Toxicity of distilled water.* Growth in distilled water was found to be more rapid than in any single-salt solution tested, and it was also more rapid than in any mixed solution containing

$\text{Ca}(\text{NO}_3)_2$  and  $\text{Mg}(\text{NO}_3)_2$ , or  $\text{KNO}_3$  and  $\text{Mg}(\text{NO}_3)_2$ . But it was slightly less rapid than in many mixed solutions containing  $\text{KNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$ , and about 18 per cent less rapid than in the standard solution, containing  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$ . These facts are evident from the data of TABLES 1-4, and FIGS.

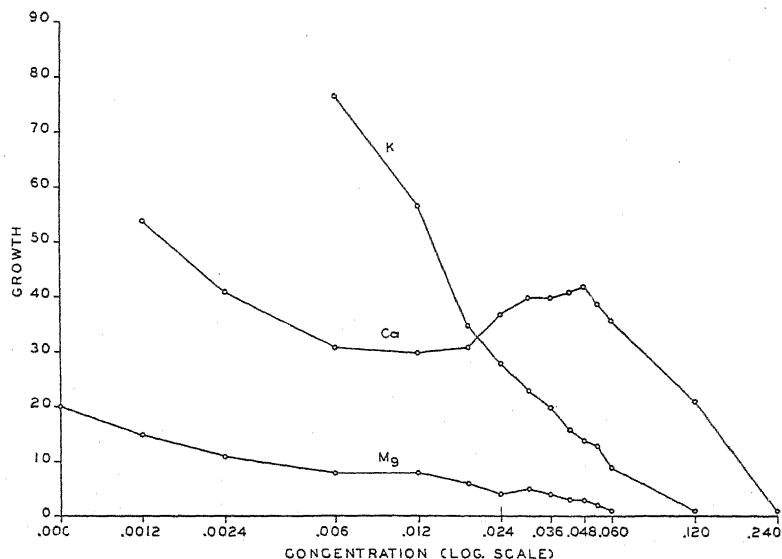


FIG. 2. Growth of wheat roots in single-salt solutions of nitrates of calcium (Ca), potassium (K), and magnesium (Mg).

Ordinates represent root growth, expressed as percentages of elongation for standard solution. Abscissas represent logarithms of volume-molecular concentrations.

1, 3, 4, and 5. The water used in the present tests was obtained from a Barnstead still. Some comparative tests indicated no significant difference between seedlings grown in this water and those grown in water redistilled from Pyrex flasks (with cotton plugs in place of cork or rubber stoppers). It is probable, therefore, that the results obtained with the various solutions of the present study are not very different from those that would have been secured if other precautions had been taken in the preparation of the distilled water used. However, on theoretical grounds, at least, water redistilled from glass (True, 1914;

Hibbard, 1915) or from quartz (Scarth, 1924) would be preferable in experiments of this kind.

In the present tests, the relatively less rapid growth in distilled water than in the standard solution and in certain two-

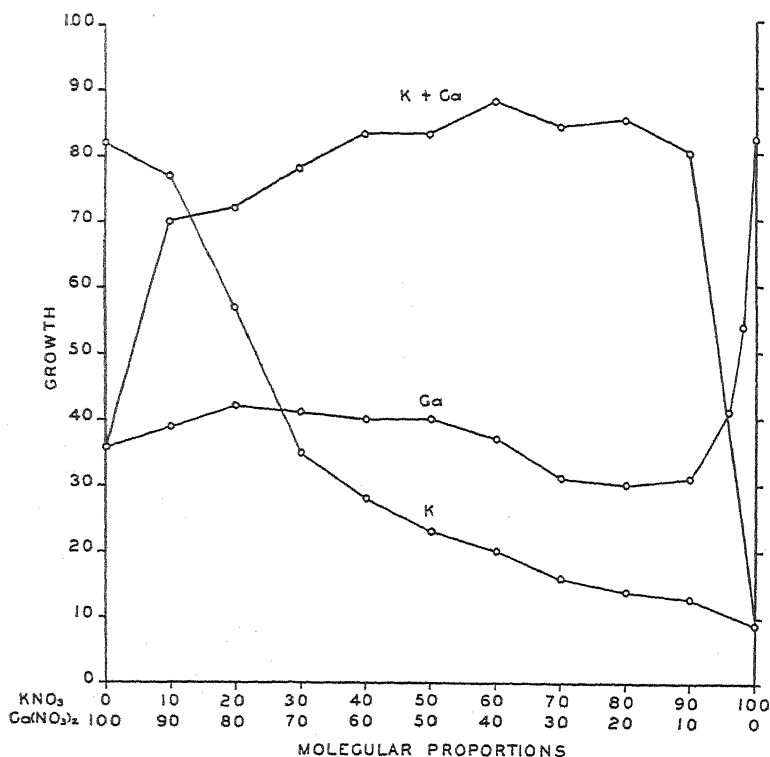


FIG. 3. Growth of wheat roots in two-salt solutions of nitrates of potassium and calcium (K + Ca), and in single-salt solutions of nitrates of potassium (K) and calcium (Ca).

Ordinates represent root growth, expressed as percentages of elongation for standard solution. Abscissas represent concentrations, expressed as percentages of 0.06 gram-molecule per liter.

salt solutions of  $\text{KNO}_3$  and  $\text{Ca(NO}_3)_2$  may perhaps be attributed to one or more of the following conditions: (1) the presence of growth-retarding substances in the distilled water, which might be antagonized by the solutes present in the standard solution, or by  $\text{KNO}_3$  plus  $\text{Ca(NO}_3)_2$ , but not by  $\text{Ca(NO}_3)_2$ ,  $\text{KNO}_3$ , or

$\text{Mg}(\text{NO}_3)_2$  alone—in the concentrations tested; (2) injurious action of distilled water due to complex effects upon permeability, such as have been suggested by True (1914); (3) so-called “nutritive effects” of the two-salt and three-salt solutions. Further study of this difficult subject is needed.

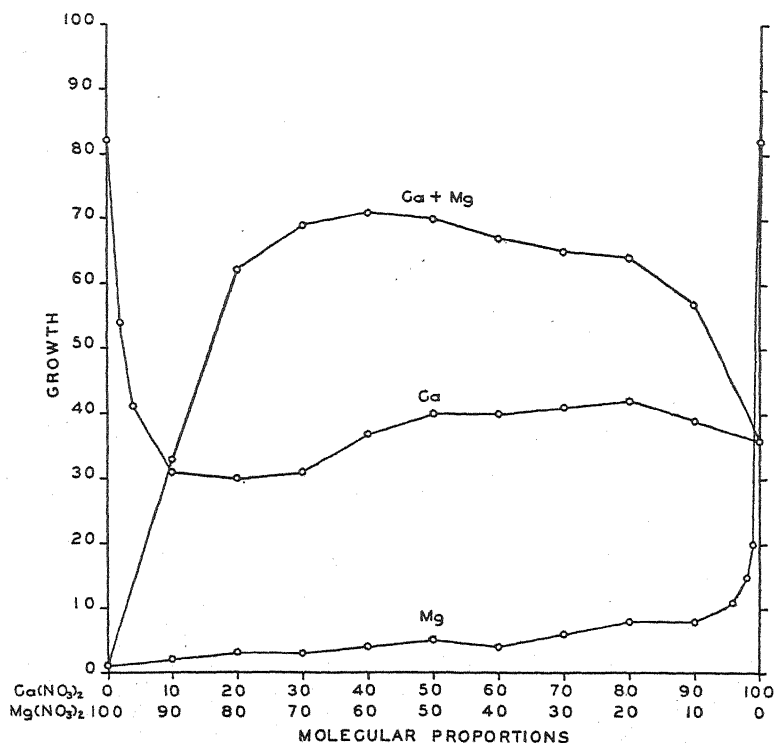


FIG. 4. Growth of wheat roots in two-salt solutions of nitrates of calcium and magnesium (Ca + Mg), and in single-salt solutions of nitrates of calcium (Ca) and magnesium (Mg).

Ordinates represent root growth, expressed as percentages of elongation for standard solution. Abscissas represent concentrations, expressed as percentages of 0.06 gram-molecule per liter.

*Peculiar rise of  $\text{Ca}(\text{NO}_3)_2$  graph.* The peculiar rise in the  $\text{Ca}(\text{NO}_3)_2$  graph deserves special attention. That this rise is significant is supported by the fact that a repetition of the test (as shown by data of TABLE I), with new solutions, gave almost exactly the same results as the first test. It is possible that this

rise in the graph may have been due to a difference in the type of response of the roots. As already mentioned, it was noted that in very low concentrations of this salt, the roots were covered with a heavy mat of long hairs, while at higher concentrations

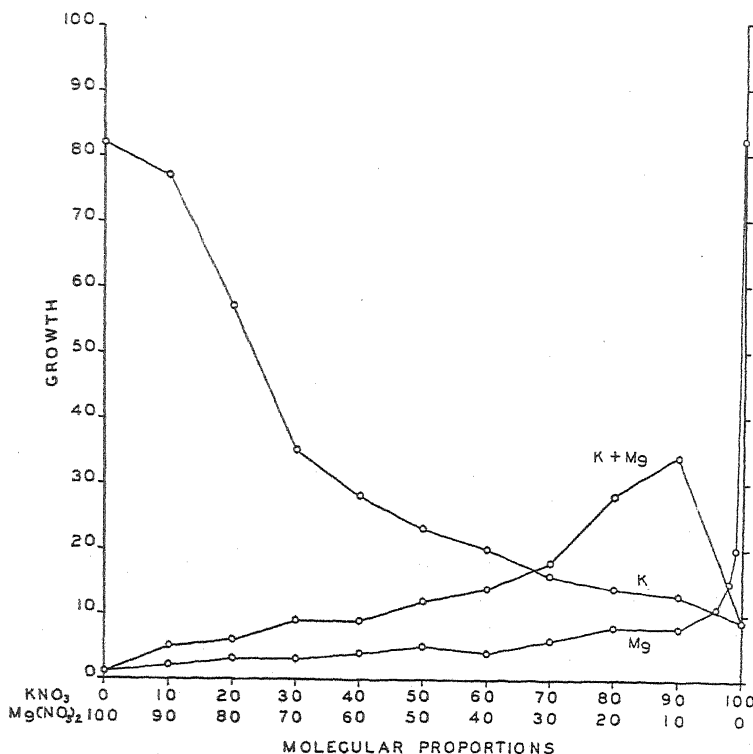


Fig. 5. Growth of wheat roots in two-salt solutions of nitrates of potassium and magnesium (K + Mg), and in single-salt solutions of nitrates of potassium (K) and magnesium (Mg).

Ordinates represent root growth, expressed as percentages of elongation for standard solution. Abscissas represent concentrations, expressed as percentages of 0.06 gram-molecule per liter.

the roots appeared more slender and hairs were much less abundant. It is possible that increase in dry weight, increase in volume, or some other criterion of growth or development, might show a simple curve such as that obtained for root growth with  $\text{KNO}_3$  and  $\text{Mg}(\text{NO}_3)_2$ . Another possible suggestion is that the fall in the graph at very low concentrations may have been



related to progressive alteration in the culture solution brought about by the plants during the period of the test. It has been noted that the plants were in many cases more variable in very dilute solutions than in more concentrated solutions. Experiments with continuously renewed solutions (Trelease and Livingston, 1922) might be used to test this possibility.

*Dissimilar effects of dilution of equally toxic solutions.* The data of TABLE 1, plotted in FIG. 1 with an ordinary scale for concentrations, have been replotted in FIG. 2, using a logarithmic scale. For any ordinate (or growth rate) the horizontal distance between two graphs represents the logarithm of the ratio of the two abscissas (or concentrations). Accordingly, if the horizontal distances between any two graphs were the same for all growth rates, this would indicate a constant ratio between the concentrations of equally toxic solutions of the two salts (using growth rates as the criterion of toxicity); or, in other words, for equal growth rates the concentration of one salt would always be a constant multiple of the concentration of the other salt. The two graphs might then be made to coincide by moving one to the right or the other to the left. If this relation existed, any pair of equally toxic single-salt solutions might be selected, and these solutions would not become unequally toxic when both were diluted to the same degree (for example, when the volume of both were doubled, tripled, etc.). Osterhout (1915) states that in most cases which have come under his observation two solutions which are equally toxic remain so (at least approximately) when both are diluted to the same degree, but he also discusses other cases that might possibly occur in which this relation would not hold.

An examination of FIG. 2 shows clearly that the horizontal distance between any pair of graphs is generally different for various growth rates. No simple constant relation exists between equally toxic concentrations of the salts in question. For these tests it is therefore evident that any pair of solutions having equal growth-retarding effects would generally have unequal growth-retarding effects when diluted to the same degree.

*Clear demonstration of antagonism.* The diagrams of FIGS. 3-5, present the results of the tests with mixed solutions, containing pairs of the salts,  $\text{KNO}_3$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{Mg}(\text{NO}_3)_2$ . Besides the graph for the growth rate in the solutions containing

the two salts, each diagram also shows the graphs for the growth rate in the simple solutions of each salt in the concentrations in which it existed in the mixed solutions.

Whether or not antagonism exists in a mixed solution of two salts (each of which would retard growth in simple solution) may in most cases be determined if we know (1) the growth rate in the mixed solution, (2) the growth rate in a simple solution of the first salt in the concentration in which it exists in the mixed solution, and (3) the growth rate in a simple solution of the second salt in the concentration in which it exists in the mixed solution. It is clear that antagonism may be considered to exist if the growth rate in the mixture is more rapid than in the simple solution having the greater growth-retarding effect.<sup>2</sup> This is the criterion of antagonism that generally has been employed by investigators, and it will be used in the present paper. Data for the necessary comparisons are available in this study, since growth data are shown, in the tables and in the graphs, for the simple solutions as well as for the mixtures.

According to the criterion here used, antagonism may be considered to exist wherever the graph representing the growth in the mixed solutions lies above the lower of the two graphs for the single-salt solutions. It will be observed that the graphs give a clear demonstration of antagonism for all tested two-salt solutions. Antagonism is especially pronounced for mixtures of  $\text{KNO}_3$  plus  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{Ca}(\text{NO}_3)_2$  plus  $\text{Mg}(\text{NO}_3)_2$ , while it is much less marked for mixtures of  $\text{KNO}_3$  plus  $\text{Mg}(\text{NO}_3)_2$ . This result is in agreement with what has generally been reported in the literature (for example, McCool, 1913).

In this connection, it is of interest to refer to methods of estimating in a quantitative manner the antagonistic effects of a mixed salt solution. As Osterhout (1914a, 1915, 1918) has shown, an accurate quantitative expression of antagonistic effects may be obtained if it is possible to estimate the total growth-retarding effect that the mixed solution would have if neither salt influenced the growth-retarding effect of the other salt. This total growth-retarding effect, Osterhout has termed

<sup>2</sup> Antagonism may exist even if the growth rate in the mixed solution is less rapid than in the more toxic single-salt solution, but it cannot be demonstrated unless an accurate calculation can be made of the "additive effect" (Osterhout, 1915, 1918) of the mixed solution.

the "additive effect." Antagonism might then be expressed accurately as the difference between the additive effect and the *actual* growth-retarding effect of the mixed solution.

If the toxic effects of the salts were exactly alike—that is, if the salts affected the same processes in the same qualitative manner—the additive effect might then be the sum of the separate toxicities of the two salts, when these toxicities were expressed in proper terms. Osterhout has shown that such a summation of the toxicities might be obtained if the effect of one salt might be expressed in terms of the other salt. For example, this might be done if the quantitative effects of the two salts were so related that, for equal growth-retarding effects, the concentration of the first salt were always a constant multiple of the concentration of the second salt. Under these conditions, equally toxic solutions of the two salts would remain equally toxic when diluted to the same degree. Osterhout (1914a) has employed a convenient method that may be used in studying antagonism between two salts which are known to be related in this manner.

As pointed out in an earlier section of this paper, equally toxic solutions of the salts here employed would not remain equally toxic when diluted to the same degree, and no constant relation is evident which would make it possible to express accurately the effect of one salt in terms of another salt. Although approximate calculations of additive effect and antagonism might be made in such cases, the simpler criterion given above has been considered adequate for the purposes of the present paper.

*Form of graphs for growth in mixtures.* It will be seen that the curves for  $\text{KNO}_3$  plus  $\text{Ca}(\text{NO}_3)_2$  and for  $\text{Ca}(\text{NO}_3)_2$  plus  $\text{Mg}(\text{NO}_3)_2$  are round-topped, with no sharp optimum set of proportions. The shape of the curve for  $\text{KNO}_3$  plus  $\text{Mg}(\text{NO}_3)_2$ , however, is different from that of the other two and indicates a rather sharply defined optimum for a mixture of 90 per cent  $\text{KNO}_3$  plus 10 per cent  $\text{Mg}(\text{NO}_3)_2$ .

The relative degree of sensitiveness of the roots to differences in salt proportions may be illustrated by the range of salt proportions in each case which allows relatively rapid growth—for example, 85 per cent or more of the maximum growth rate. The following tabulation shows this range for each mixture:

*From 25 per cent  $\text{KNO}_3$  + 75 per cent  $\text{Ca}(\text{NO}_3)_2$  to 91 per cent  $\text{KNO}_3$  + 9 per cent  $\text{Ca}(\text{NO}_3)_2$ .*

*From 19 per cent  $\text{Ca}(\text{NO}_3)_2$  + 81 per cent  $\text{Mg}(\text{NO}_3)_2$  to 86 per cent  $\text{Ca}(\text{NO}_3)_2$  + 14 per cent  $\text{Mg}(\text{NO}_3)_2$ .*

*From 81 per cent  $\text{KNO}_3$  + 19 per cent  $\text{Mg}(\text{NO}_3)_2$  to 92 per cent  $\text{KNO}_3$  + 8 per cent  $\text{Mg}(\text{NO}_3)_2$ .*

Thus 85 per cent or more of the maximum growth rate was secured with 66 per cent of the possible range of proportions of  $\text{KNO}_3$  plus  $\text{Ca}(\text{NO}_3)_2$ , with 67 per cent of the range of proportions of  $\text{Ca}(\text{NO}_3)_2$  plus  $\text{Mg}(\text{NO}_3)_2$ , but with only 11 per cent of the range of proportions of  $\text{KNO}_3$  plus  $\text{Mg}(\text{NO}_3)_2$ .

Molecular ratios may be used similarly. For solutions giving 85 per cent or more of the maximum growth rate, the values of the ratio of  $\text{KNO}_3$  to  $\text{Ca}(\text{NO}_3)_2$  range from 0.33 to 10.1, those of the ratio of  $\text{Ca}(\text{NO}_3)_2$  to  $\text{Mg}(\text{NO}_3)_2$  range from 0.23 to 6.1, and those of the ratio of  $\text{KNO}_3$  to  $\text{Mg}(\text{NO}_3)_2$  range from 4.3 to 11.5.

The present data suggest that for a total molecular concentration of 0.06 M, the two-salt solutions which would allow most rapid growth might have approximately the following molecular proportions: (1) 60 per cent  $\text{KNO}_3$  plus 40 per cent  $\text{Ca}(\text{NO}_3)_2$ , (2) 40 per cent  $\text{Ca}(\text{NO}_3)_2$  plus 60 per cent  $\text{Mg}(\text{NO}_3)_2$ , (3) 90 per cent  $\text{KNO}_3$  plus 10 per cent  $\text{Mg}(\text{NO}_3)_2$ . But careful tests with large numbers of wheat seedlings would be necessary to determine with any high degree of accuracy the exact proportions of the optimum solutions, for any given set of non-solution conditions.

The wide range of salt proportions (or molecular ratios) allowing approximately optimum growth is one of the outstanding features of the results here reported. Earlier investigations of two-salt solutions, where actual data have been given, have generally indicated much narrower limits for this range. For example, the results of Osterhout (1909) for concentrated solutions indicate sharply defined optimum salt proportions. The difference in question appears to be related to the total concentrations employed. For any pair of salts, the form of the growth curve may be expected to be greatly influenced by the total concentration of the solution. Thus, Osterhout (1914b) gives diagrams showing how the form of the growth curve for mixed solutions is altered with dilution of the latter. He states that with dilution the growth curve becomes flatter, and that at great

dilution it must tend to become a horizontal straight line. As the concentration is lowered, the growth-retarding effect diminishes and its inhibition is consequently less striking (Osterhout, 1908).

In each diagram the abscissa corresponding to the point of intersection of the graphs for the single-salt solutions indicates the mixed solution composed of equally toxic concentrations of the two constituent salts. These points are approximately as follows:

27 per cent  $\text{KNO}_3$  + 73 per cent  $\text{Ca}(\text{NO}_3)_2$ ,  
 99 per cent  $\text{Ca}(\text{NO}_3)_2$  + 1 per cent  $\text{Mg}(\text{NO}_3)_2$ ,  
 96 per cent  $\text{KNO}_3$  + 4 per cent  $\text{Mg}(\text{NO}_3)_2$ .

Mixed solutions to the left of this point, in each case, are ones in which the first salt may be regarded as existing in the less toxic concentration, while mixed solutions to the right are ones in which the second salt may be considered as existing in the less toxic concentration.

It will be noted that for each pair of salts the graph representing the growth rate in the mixed solutions lies entirely above the lower of the two graphs for the single-salt solutions. Thus the growth in every two-salt solution is more rapid than in the corresponding simple solution of the constituent salt existing in the more toxic concentration.

Moreover, for each pair of salts the graph representing the growth rate in the mixed solutions lies, for a considerable portion of its range, above the higher of the two graphs for the single-salt solutions. The following summary shows the range of salt proportions including every two-salt solution in which the growth is more rapid than in the corresponding simple solution of the constituent salt existing in the less toxic concentration:

From 13 per cent  $\text{KNO}_3$  + 87 per cent  $\text{Ca}(\text{NO}_3)_2$  to 96 per cent  $\text{KNO}_3$  + 4 per cent  $\text{Ca}(\text{NO}_3)_2$ .

From 10 per cent  $\text{Ca}(\text{NO}_3)_2$  + 90 per cent  $\text{Mg}(\text{NO}_3)_2$  to 99 per cent  $\text{Ca}(\text{NO}_3)_2$  + 1 per cent  $\text{Mg}(\text{NO}_3)_2$ .

From 68 per cent  $\text{KNO}_3$  + 32 per cent  $\text{Mg}(\text{NO}_3)_2$  to 98 per cent  $\text{KNO}_3$  + 2 per cent  $\text{Mg}(\text{NO}_3)_2$ .

Thus 83 per cent of the possible total range of mixtures is included for the first mixture, 89 per cent for the second mixture, but only 30 per cent for the third mixture. Within these limits, antagonism is shown in a striking manner, and the salts are seen to be mutually antagonistic.

*Toxicity of mixtures.* It is interesting to compare the maxi-

imum growth rates for the three pairs of salts. Growth in the best solution containing  $\text{KNO}_3$  plus  $\text{Ca}(\text{NO}_3)_2$  was 88 per cent as rapid as in the three-salt solution (containing  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$ ) used as the standard solution and was more rapid than in distilled water (the latter allowing a growth rate 82 per cent as rapid as in the standard solution). The best solution containing  $\text{Ca}(\text{NO}_3)_2$  plus  $\text{Mg}(\text{NO}_3)_2$  allowed almost as rapid growth (71 per cent of the rate for the standard solution), while the best solution containing  $\text{KNO}_3$  plus  $\text{Mg}(\text{NO}_3)_2$  permitted much less rapid growth (only 34 per cent of the rate for the standard solution). These general relations might be expected from the relative growth-retarding effects of the single-salt solutions,  $\text{Mg}(\text{NO}_3)_2$  generally having a greater retarding effect than  $\text{KNO}_3$ , and the latter a greater retarding effect than  $\text{Ca}(\text{NO}_3)_2$ . Combinations of the two more toxic salts ( $\text{KNO}_3$  and  $\text{Mg}(\text{NO}_3)_2$ ) give greater growth retardation than combinations of the most toxic salt ( $\text{Mg}(\text{NO}_3)_2$ ) with the least toxic salt ( $\text{Ca}(\text{NO}_3)_2$ ), and the latter give greater retardation than combinations of the two less toxic salts ( $\text{KNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$ ).

#### SUMMARY

A study was made of the rate of elongation of wheat roots immersed in simple solutions of potassium nitrate, calcium nitrate, and magnesium nitrate, and in solutions containing pairs of these salts. The single-salt solutions included a wide range of concentrations. Every two-salt solution had a total concentration of 0.06 gram-molecule per liter, but in the various solutions of each series the volume-molecular partial concentrations of the constituent salts were varied in a systematic manner.

The results of this study indicate that very young wheat seedlings can be used satisfactorily for demonstrating the growth-retarding effects of single-salt solutions and the antagonistic action of mixed solutions.

The relative degrees of toxicity of the three salts (as indicated by growth-retarding effect) were found to be in general agreement with most published data. For equal molecular concentrations, magnesium nitrate was much more toxic than either potassium nitrate or calcium nitrate. It was found that at low concentrations potassium nitrate was less toxic than calcium nitrate, while at higher concentrations the reverse was true.

By means of graphs in which a logarithmic scale is used for concentrations, it is shown that, for these salts and conditions, equally toxic simple solutions of different salts usually become unequally toxic when these solutions are diluted to the same degree.

Antagonism was found to be especially pronounced for mixed solutions of potassium nitrate and calcium nitrate, and of calcium nitrate and magnesium nitrate, but it was much less marked for mixed solutions of potassium nitrate and magnesium nitrate.

Some striking differences in appearance of roots grown in single-salt solutions are described, which indicate that each salt has specific toxic effects.

Methods of measuring antagonism in a mixed salt solution are briefly discussed, with special reference to the assumptions involved.

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## On *Lupinus fraxinetorum* Greene

WILLIAM A. DAYTON

(WITH PLATE 5 AND TWO TEXT FIGURES)

*Lupinus fraxinetorum* was published by the late Dr. Edward L. Greene in Leaflets, Vol. II, pp. 234-5, 1912, and is founded on Mr. Ralph Hopping's no. 8, Forest Service serial no. 3055, collected in 1911 in Fresno County, California, on the Sequoia National Forest. The type is in the U. S. National Herbarium, but part of the type material (the 'isotype,' to use Dr. Pennell's convenient term) is deposited in the herbarium of range plants in the Washington office of the Forest Service. This isotype is illustrated herewith in PLATE 5. Unfortunately the indument on the stem (and, to a lesser extent, on the leaflets) of conspicuous long, not dense, spreading, multicellular hairs, which is so conspicuous a feature of these specimens, is not brought out in the photograph. These hairs have the fulvous aspect that is often observed, for example, in much California herbarium material labeled *Lupinus leucophyllus*.

It is not for the present writer to comment concerning the the specific status of *Lupinus fraxinetorum*; its adequate diagnosis must be left to special students of this difficult genus, such as Professors Robinson and C. Piper Smith, Mr. Eggleston, and Dr. Rydberg. Suffice it for the nonce to state that the plant possesses 'an aspect,' and, while the accompanying illustrations (PLATE 5, and FIG. 1, b) fail to show, for example, the glabritty of the subrhomboid banner (an item not alluded to in Dr. Greene's original diagnosis of the species) yet PLATE 5 does indicate clearly the ample free stipules to which the author of the species refers.

The writer wishes to call attention to the fact that it was the intention of Dr. Greene to name this plant *Lupinus fraxineus* and that the specific name, '*fraxinetorum*,' which the latter published for the species was a slip-of-the-pen and a misnomer. PLATE 5 and FIG. 2 herewith both indicate plainly, in Dr. Greene's own handwriting, that *Lupinus fraxineus* was the name which he intended to bestow upon it. In 1912 Dr. Greene visited my office to notify me of his interest in Forest Service specimen no. 3055, which (as was his wont in such cases) he designated as

"New to me, hence new to Science!" He told me he had bestowed the name of *Lupinus fraxineus* on this new lupine, stating, in his characteristic fashion, that "Many botanists would dub such a plant '*Lupinus fresnoensis*,' a clear barbarism, which one familiar with the Spanish etymology of Fresno (California) would not perpetrate."

It was evident from the start that Dr. Greene regarded this plant not at all as one growing in ash woods as the specific

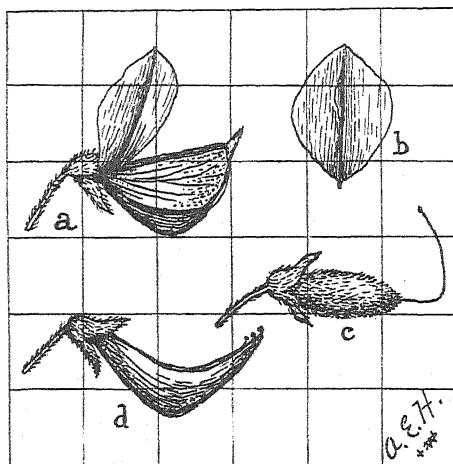


FIG. 1. Some floral aspects of *Lupinus fraxineus* Greene: (a) Flower; (b) Banner, or standard; (c) Immature pod, showing persistent, reflexed and geniculate style; (d) Keel, undetached from calyx.

The background is of 9 centimeters square, the small squares 5 mm. square, all  $\times 2$ .

name *fraxinetorum* would indicate,<sup>1</sup> but as being connected geographically with Fresno County, and he told me, with visible embarrassment after the specific name *fraxinetorum* appeared in print, that that was not the name he intended to publish. In fact, the concluding sentence of his original description clearly demonstrates that a mnemonic lapse resulted in the published name *fraxinetorum*.

The writer of this article maintains that *Lupinus fraxineus* Greene is the rightful name of this lupine, and that *L. fraxinetorum* is a usurper and can only technically be maintained. He

<sup>1</sup> Note in PLATE 5 that Mr. Hopping collected this plant in yellow pine timber.

would like to urge upon the nomenclatorial pundits of the botanical world the adoption of some ruling whereby names that are clearly slips-of-the-pen, misnomers unintended by their makers, should be suppressed in favor of the respective names

SMITHSONIAN INSTITUTION

UNITED STATES NATIONAL MUSEUM

MEMORANDUM

This cover contained a mounted  
sheet of excellent specimens of  
a lupine quite new to science  
and to be named

Lupinus fraxinus

The collector's number is

The plant is said to be common  
in pine woods. More specimens  
should be obtained the coming  
season. It is a very interesting  
specimen botanically.

Chas. L. Greene

officer

FIG. 2. The memorandum, in Dr. Greene's own hand, which he put in the folder of Forest Service specimen no. 3055, from which he had withdrawn the type material of his *Lupinus fraxineus*. Note especially the form of the specific name. The original memorandum is filed with the isotype in the range herbarium of the Forest Service, Washington, D. C.

wherewith those authors obviously intended to christen their plant children.

I have another analogous case of this sort just at present in mind. In his original description of *Hulsea nana* (Pacific Railroad Report, Vol. 6, Pt. 3, p. 76, 1857) Dr. Gray has this noteworthy sentence: "This is one of the most interesting plants

of Dr. Newberry's collection, and I have great pleasure in proposing that the species shall bear his name." Unless, indeed, one maintains the incredible thesis that the illustrious Asa Gray gave expression here to a most extraordinary bit of satire or sardonic humor, one cannot possibly conclude that *Hulsea nana* bears the name of its type collector, Dr. Newberry, or in any way honors him, *or that it is the name which Dr. Gray intended to confer upon the species*. Therefore, there is no doubt in the writer's mind that *Hulsea Newberryi* A. Gray is the *de veritate* (and I trust some day the *de jure factoque*) name of what is now called *Hulsea nana*.

I respectfully commend the above comments to the earnest meditation of those who will control and advise future nomenclatorial conventions.

#### Description of plate 5

The isotype of *Lupinus fraxineus* in the range herbarium of the Forest Service Washington, D. C. Note Dr. Greene's original identification slip.





## Notes on Fabaceae—VII

PER AXEL RYDBERG

### GEOPRUMNON Rydberg

The genus was described (Small, Fl. S. E. U. S. 615, 1332. 1903) from five species of *Astragalus*, of which *A. crassicaarpus* Nuttall was regarded as the type. It corresponds to Gray's section SARCOCARPI and is characterized by the thick, fleshy, 2-celled pod, becoming spongy in age and tardily dehiscent, many-seeded. The genus is wholly North American and consists of the following species.

Pod glabrous, at least in age.

Pod subglobose or broadly ellipsoid, abruptly mucronate at the apex.

Calyx strigose.

Pod subglobose, about as broad as long, 2-2.5 cm. long; corolla about 2 cm. long.

Corolla purple; leaflets oblong to linear.....1. *G. crassicaarpus*

Corolla cream-colored with purple keel; leaflets oval or obovate.....2. *G. succulentum*

Pod broadly ellipsoid, 2.5-3 cm. long; corolla about 3 cm. long, white or ochroleucous .....3. *G. mexicanum*

Calyx densely woolly; corolla ochroleucous; pod subglobose.....4. *G. trichocalyx*

Pod obliquely ovoid, acuminate; corolla ochroleucous; leaflets lance-oblong .....5. *G. pachycarpum*

Pod pubescent.

Pod somewhat oblique but not curved, less than 2 cm. long, short-acuminate; stems with short ascending hairs .....6. *G. plattense*

Pod arcuate, more than 2 cm. long, gradually acuminate; stem with long spreading hairs .....7. *G. tennesseense*

1. *GEOPRUMNON CRASSICARPUM* (Nutt.) Rydb. *Astragalus crassicaarpus* was described by Nuttall in Frazer's Catalogue of 1813. The description is quite inadequate, consisting only of the following: "† Fruit about the size and form of *A. physodes*, but thick and succulent. Collected above the Platte River." [The sign † stands for perennial.] There is, however, no uncertainty about the identity of the plant as it is the only species which grows in the region of Nuttall's travels along the Missouri River and which fits this description. *A. carnosus* Pursh was

described from specimens collected in "Upper Louisiana" by Bradbury, who accompanied Nuttall on the trip mentioned. Bradbury's specimens consisted of flowers of *Sophora sericea* and fruit of *A. crassicaarpus*. Pursh also compares the fruit with that of *A. physodes*. The first complete description was that by Kerr in the Botanical Register, *plate 176*, under the name of *A. caryocarpus*. It was also illustrated on *plate 1324* of the same work under the name of *A. succulentus* Richards. See next species. The species grows on the prairies and plains from Manitoba to Missouri, Tennessee, Texas, New Mexico, Montana, and Saskatchewan.

2. *GEOPRUMNON SUCCULENTUM* (Richards.) Rydb. *Astragalus succulentus* Richardson was collected on Franklin's first journey. There is some doubt as to the identity of this species, as Richardson did not have mature fruit, but it cannot be the species illustrated under that name in the Botanical Register, *plate 1324*, for the plate illustrates a purple-flowered plant, evidently the same as *A. caryocarpus* Kerr (see above); while Richardson described his plant as having ochroleucous corolla with the keel purple. Thinking that the plate mentioned represented the original *A. succulentus* Richardson, I redescribed the present species under the name *A. prunifer*. It differs from *G. crassicaarpus* besides in the color of the flower, in the lighter green foliage, the broader leaflets and the usually somewhat larger flowers and fruit. Webber in his Catalogue of the Flora of Nebraska mistook it for *A. mexicanus* A. DC., under which name it appeared in my treatment of the Flora of Nebraska. In fact it is more closely related to that species than is *A. trichocalyx* Nutt., to which Gray applied the name *A. mexicanus*. It is distributed from Saskatchewan and Alberta to Colorado.

3. *GEOPRUMNON MEXICANUM* (A. DC.) Rydb. *Astragalus Mexicanus* A. DC. was described from specimens collected in Texas by Berlandier. Torrey & Gray in their flora, page 693, referred this to *A. trichocalyx* Nutt., and Gray (Proc. Am. Acad. 6: 193. 1863) formally adopted *A. mexicanus* for the latter. Botanists in general have followed their interpretation, but neither Berlandier's specimens nor A. de Candolle's plate warrant it; neither illustrates a plant with a woolly calyx. In Can-



dolle's diagnosis, the fruit is described as being glabrous, but the plate shows a few hairs. The fruit, as illustrated, differs from that of both *A. crassicaarpus* and *A. trichocalyx* Nutt., in being larger and more elongate. A good illustration is given by Jones in his Revision of *Astragalus* under the name *A. crassicaarpus pachycarpus*. *Berlandier* 358 has a fruit 3 cm. long and 1.15 cm. in diameter, and perfectly glabrous. Heller's specimen from Kerrville has one 3.5 cm. long and 2 cm. thick. The flower resembles mostly that of *G. plattense* but is larger and with longer calyx-teeth. In *Lindheimer* 597 the corolla is 28 mm. long. This number was rightly referred to *A. mexicanus* in *Plantae Lindheimerianae* by Dr. Gray, but later he has written on the label "*A. Plattensis* Nutt. wrongly referred in Pl. Lindh. to *A. mexicanus*." In *Plantae Wrightianae* he transferred this number to *A. plattensis*. His statement that the ovary is pubescent is so far correct that it is minutely puberulent. The fruit of *Berlandier* 358 is, however, quite different from that of *G. plattense*, twice as long, at least 3 times as broad in the median plane, and not acuminate. The following specimens belong here.

TEXAS: *Berlandier* 358; 273; *Lindheimer* 597, 200, 230, in part; *Wright* in 1848; Kerr Co., *Bray* 219; *Heller*, in 1894; Bexar Co., *Ellen Schulz* 298; 23.—KANSAS: Cherokee Co., *Hitchcock* 1015.—? NEBRASKA: Hyannis, *Morris* 518. These specimens are doubtful, being in fruit and leaves mostly gone; but the large fruit indicates this species.

4. ***Geoprumnon trichocalyx* (Nutt.) Rydb.** *Astragalus trichocalyx* Nutt. T. & G. Fl. N. Am. 1: 322. 1838. *Astragalus mexicanus* A. Gray, Pl. Wright 1: 51, in part. 1852. *Geoprumnon mexicanum* Rydb.; Small. Fl. S. E. U. S. 616, 1332, in part. 1903. Outside of the confusion in nomenclature (in its being mistaken for *A. mexicanus*), this species has been well understood.

ILLINOIS: Bluffs, *Eggert*, in 1875; Joliet, *E. J. Hill*, in 1902.—MISSOURI: *Riehl* 330; St. Louis, *Halsted* & *Engelmann*; *Engelmann*, in 1832; *Geyer*, in 1842; *Eggert*, in 1875 and 1877; Jefferson Co., *Hasse*, in 1887; Montier, *Bush* 1486, 6654; Raynolds, *Eggert*, in 1887; Allenton *Letterman*, in 1912; St. Louis Co., *Glatfelter*, in 1894; Willard, *Blankinship*, in 1887.—OKLAHOMA: Vinita, *Carleton*, 38, in 1891.—LOUISIANA: *Hale*.—ARKANSAS:

*Nuttall*; Fayetteville, *Wells* 29.—TEXAS: *Wright* (in fruit); *Lindheimer* 230, in part; *E. Meyer*, in 1844; *Eggert*, in 1897.

5. *GEOPRUMNON PACHYCARPUM* (T. & G.) Rydb. *Astragalus pachycarpus* was described from fruiting specimens collected by Leavenworth. Dr. Gray regarded it as a depauperate form of *A. caryocarpus* (i. e. *G. crassicaupum*); but the fruit is that of *G. plattense*, though glabrous. The leaflets, however, are narrower and inclined to be acutish and the pubescence is more appressed. I have seen no specimens with mature fruit except the type. There are, however, flowering specimens and specimens in young fruit which have narrow leaflets. The former have the flowers of *G. plattense* rather than *G. crassicaupum*. The specimens with young fruit might belong to *G. crassicaupum*, but I am inclined to refer them here. If *A. pachycarpus* should be reduced, it should be to a variety of *G. plattense*, not to *G. crassicaupum*, as Jones has done. Some of the specimens cited by him (*Contr. W. Bot.* 8: 17. 1898) evidently belong here, but the illustration of the pod in his Revision, evidently belongs to *G. mexicanum*. On the other hand one of the fruits (the glabrous one) intended to represent *A. plattensis*, looks more like that of the type of *A. pachycarpus*.

TEXAS: *Lindheimer* 596; 745, 744; San Filipe de Austin and San Patricio, *E. Meyer*, in 1844; Bexar County, *Jermy* 17.—ARKANSAS: *Leavenworth*.—? ARIZONA: *C. D. Marsh* 14242 (*U. S. Nat. Herb.* 1117846). This specimen was collected in fruit only and is doubtful. The pods being more abruptly contracted at the apex and the epicarp thinner.

6. *GEOPRUMNON PLATTENSE* (Nutt.) Rydb. This has ovoid, acuminate, strigose pods, and rather small ochroleucous or cream-colored corollas, only the keel tipped with purple. Its distribution extends from Indiana (?) to Minnesota and the Black Hills (South Dakota), south to Texas and Alabama.

7. *GEOPRUMNON TENNESSEENSE* (A. Gray) Rydb. This differs from the rest of the species in its long villous stem and pod and in the pod being lanceolate in outline.

TENNESSEE: Glades, *Gattinger*, in 1878, 1880 and 1881, also (*Curtis' no.*) 593; Nashville, *Dr. Roane*, *Bicknell*; *Gattinger Hubbard*; *Lesquereux*; *Rutherford*; *Eggert*, in 1898.—ALABAMA: *Hatch*, in 1854.—ILLINOIS: *Ottawa*, *Bebb* in 1897 (This spe<sup>c</sup>

men has narrow leaflets and stipules); Ogle Co., *Bebb*; Morris, *Vasey*, in 1861; Marseilles, *Johnson*, in 1900.

### HESPERASTRAGALUS Heller

The genus was established by Heller (*Muhlenbergia* 2: 87. 1905) and based on Gray's section DIDYMOCARPI of *Astragalus*. Jones extended Gray's section so as to include also the REFLEXI. This disposition I am inclined to follow. *Astragalus Breweri*, however, included by Jones, does not belong here. Heller's diagnosis of the genus must therefore be somewhat modified.

Pod decidedly longer than broad, broadest below the middle and tapering at the apex. .... 1. REFLEXI

Pod about as broad as long or broader, rounded and merely mucronate at the apex.

Body of the pod fully 5 mm. long ..... 2. DIPHACI

Body of the pod about 3.5 mm. long or less. .... 3. DIDYMOCARPI

#### I. REFLEXI

Annual; leaflets obovate, deeply retuse at the apex ..... 1. *H. reflexus*

Perennial with a creeping rootstock; leaflets linear-oblong ..... 2. *H. oxyrhynchus*

1. ***Hesperastragalus reflexus*** (T. & G.) Rydb. *Astragalus reflexus* T. & G. Fl. N. Am. 1: 334. 1838. In this species the ovules are 3 or 4 in each cavity, while in most of the species only one or two. The type was collected by Drummond in Texas.

TEXAS: *Drummond* 140; Dallas, *Reverchon* 263; 243; 594 (Curtis' no.); Manor, *E. Hall* 151; Austin, *Young*, in 1914.

2. ***Hesperastragalus oxyrhynchus*** (Hemsley) Rydb. *Astragalus oxyrhynchus* Hemsley, Biol. Cent. Am. Bot. 1: 265. 1878. The type was collected at Tizapan, as given by Hemsley, or Zapan as given on the label, in the Federal District of Mexico, by Bourgeau (no. 329). The isotype of this number in the Gray Herbarium is in flowers only, but the pod is described as being acute. Good fruiting specimens are represented by *Pringle* 9722, and correctly labeled. Jones described *A. angelinus* from specimens from near San Angel in the valley of Mexico, *Rose and Painter* 6490. This I am inclined to refer here. Jones described the fruit as being "obtuse or a little retuse." In the isotype in the herbarium of the New York Botanical Garden, some of the pods are decidedly acute, while others are obtuse and mucronate.

The latter are evidently not normal. In another number of *A. angelinus*, cited by Jones, *Rose & Painter* 7090, the pods are exactly like those of *Pringle* 9722. Jones placed *A. angelinus* next to *A. diphacus*, while he placed *A. oxyrhynchus* in the MICRANTHI. They are evidently the same.

MEXICO (Federal District): Zapan, *Bourgeau* 329; San Angel, *Rose & Painter* 6490.—HIDALGO: El Salto, *Pringle* 9722; Tula, 6404. *Rose & Painter* 7090.

#### DIPHACI

Pod strigulose, subsessile; leaflets linear, obtuse or acutish. . . . . 3. *H. diphacus*

Pod glabrous, distinctly stipitate, the stipe about equalling the calyx; leaflets obovate, retuse. . . . . 4. *H. brazoensis*

3. ***Hesperastragalus diphacus*** (S. Wats.) Rydb. *Astragalus diphacus* S. Wats. Proc. Am. Acad. 17: 342. 1882. This was described from *Schaffner* 816 from San Luis Potosi. It resembles the preceding species a good deal, but the pod is different, being rounded or retuse at the apex, didymous, the axis of the cells forming a straight line.

SAN LUIS POTOSI: *Schaffner* 612/816.—ZACATECAS: *Pringle* 1753.—MEXICO: [without locality] *Woelffin*, in 1845.—JALISCO: Constanica, *Pringle* 11362.

4. ***Hesperastragalus brazoensis*** (Buckley) Rydb. *Astragalus brazoensis* Buckley, Proc. Acad. Phila. II. 5: 452. 1861. This species resembles *H. reflexus* in habit but the pod is distinctly stipitate and broader than long. The type came from "Western Texas" [more properly Eastern Texas, if it was named for Brazos River.]

TEXAS: *Buckley*; Corpus Cristi Bay, *Heller* 1483; Valley of the Nueces, *Major Thomas*; *Palmer* 244; Eagle Pass, *H. C. Hanson* 690; San Antonio *Rockwell*, in 1912.—TAMAULIPAS: Matamoros, *Tracy* 9091.

#### DIDYMOCARPI

Calyx white-hairy.

Pod short pubescent, strongly rugose; leaflets more or less

cuneate, deeply retuse. . . . . 5. *H. dispermus*

Pod glabrous, not strongly rugose, leaflets oblong, not retuse.

6. *H. obispensis*

Calyx black-hairy.

Pod pubescent; corolla less than 7 mm. long.

Leaflets oblong or cuneate, not deeply emarginate.

Pod densely pubescent with appressed hairs, not strongly rugose; racemes elongate, linear..... 7. *H. Elmeri*

Pod with spreading pubescence; raceme short, rounded to oblong.

Pod rather densely long-hairy; corolla 4-5 mm. long.

Flowers subsessile in a dense head; pod not reflexed.

8. *H. compactus*

Flowers short pedicelled; raceme in fruit slightly

elongate; pod reflexed..... 9. *H. Gambellianus*

Pod short-pubescent; corolla 6-7 mm. long..... 10. *H. didymocarpus*

Leaflets linear, usually deeply emarginate..... 12. *H. catalinensis*

Pod glabrous, corolla about 10 mm. long..... 11. *H. Milesianus*

5. *HESPERASTRAGALUS DISPERMUS* (A. Gray) Heller. Jones in his Revision distinguished this species from *H. didymocarpus* by the pod, which according to him is "3-3 [evidently he means 3-4] mm. long, with cross-section flatly triquetrous-cordate" in the former, and "3 mm. long, with cross-section a crescent," in the latter. He figured both as obcordate, but that of *A. dispersus* slightly more broadly so. In fact there is little difference in the fruit, though that of *H. didymocarpus* is somewhat broader. In *H. dispersus* the calyx is white-hairy with shorter teeth and the leaflets comparatively shorter and broader; in *H. didymocarpus* the calyx is black-hairy especially when young.

ARIZONA: Wickerberg, *Palmer* (type); Apache Gap, *Lemmon* 606; Verde River, *Smart* 150; Tucson, *Griffiths* 3638; Mohave Desert, *Lemmon*, in 1884.—CALIFORNIA: San Diego County, *Pringle*, in 1882; *Abrams* 3568; San Felipe, *Brandeggee*, in 1891; Elsenor, *MacClatchie*; Palm Springs; *Grant*, in 1906; *Eastwood* 2601; 3058; Cariso Creek, *Brandeggee*, in 1905; San Bernadino County, *Parish* 19242; *Purpus* 5553; Warner's Hot Springs, *Eastwood* 2601; Palm Spring, *H. M. Hall* 5763.—LOWER CALIFORNIA: Hansen's, *Orcutt*; Todos Santos Bay, *Jones*, in 1882.

6. *Hesperastragalus obispensis* Rydb., sp. nov. An annual; stem 1 dm. high, branched at the base, the branches ascending or spreading, minutely strigose; leaves 2-3 cm. long, spreading; stipules herbaceous, deltoid, acuminate, 2 mm. long; leaflets 9-11, elliptic or oblong, mostly rounded at the apex, 4-5 mm. long, pilose on both sides; peduncles 2-4 cm. long, slender; raceme dense, head-like, 1.5-2 cm. long, 1.5 cm. in diameter, bracts subulate, 2-3 mm. long; flowers nearly sessile; calyx white-pilose, the tube broadly campanulate, 2 mm. long, the teeth subulate, 2 mm. long; corolla apparently white with purple-tipped keel, 5-6 mm.

long; banner with an oval blade, strongly arcuate; wings shorter, the blades obliquely oblanceolate; keel-petals nearly as long, the blade lunate, strongly arched at the middle, attenuate at the apex; pod glabrous, 3 mm. long, about as broad, 2 mm. deep, broadly ovate when seen from above, glabrous, acute.

The type was collected at San Luis Obispo, California, May 6, 1882, *Jones 3229* (in part, herb. N. Y. Bot. Gard.). The sheet contains 3 specimens. It was first labeled *Astragalus didymocarpus*, then corrected to *A. dispermus*. The lower two specimens belong to this species, the upper and larger one is a specimen of *H. Gambellianus*. Two other sheets of the same number are also in the herbarium of the New York Botanical Garden. Jones has corrected the determination of one to *A. dispermus*, the other to *A. nigrescens*. Both represent *H. Gambellianus*; so also the sheet in the National Herbarium.

7. **Hesperastragalus Elmeri** (Greene) Rydb. *Astragalus Elmeri* Greene, *Erythraea* 3: 98. 1895. This differs from its closest relatives in the more elongate inflorescence and its appressed-hairy fruit. To it belongs the following specimens:

CALIFORNIA: Ros Valley, *Elmer Drew* (type); Stanford University, *Baker 718* (determined as *A. catalinensis*); *Heller 7357* (as *A. nigrescens*); *Elmer 4636* (as *A. didymocarpus*).

8. **HESPERASTRAGALUS COMPACTUS** Heller. Jones reduced this to a synonym of *A. dispermus*, but it is evidently closer to *H. Gambellianus* (*A. nigrescens* Nutt.), having the black-hairy calyx. I have seen specimens only of the type collection, *Heller 8156*, from Pollasky, Fresno County, California.

9. **HESPERASTRAGALUS GAMBELLIANUS** (Sheldon) Heller. This was first described as *Astragalus nigrescens* Nutt., but the name was changed to *A. Gambellianus* by Sheldon, as there was an older *A. nigrescens* Pallas.

OREGON: Ashland, *Howell*, in 1899.—CALIFORNIA: numerous specimens.—LOWER CALIFORNIA: Encenada, *Jones*, in 1882.

10. **HESPERASTRAGALUS DIDYMOCARPUS** (H. & A.) Heller. This is well understood.

CALIFORNIA: Douglas; Santa Barbara, *Elmer 3838*; Tracy; San Joaquin County, *Baker 2782*; Kern County, *Heller 7600*; Antioch, *Brandeggee*; Mojave, *Mrs. De Kalb*; San Clemente Island,

*Trask 206*; San Bernadino Co., *Parish 5560*; San Joaquin Valley, *Burt Davis 3062*.

11. *Hesperastragalus Milesianus* Rydb., sp. nov. Annual; stem erect, 2-3 dm. high, glabrous or with a few scattered hairs; leaves 4-6 cm. long, ascending; stipules 2-3 mm. long, deltoid, more or less scarious; leaflets 11-13, oblong-cuneate, about 1 cm. long and 2 mm. wide, emarginate, glabrous above, sparingly hairy beneath; peduncles 4-5 cm. long; raceme head-like, dense, 1-2 cm. long, fully 1 cm. broad; bracts lanceolate, 1-2 mm. long; flowers subsessile; calyx black-hairy, the tube 2-2.5 mm. long, the teeth subulate, fully 1 mm. long; corolla purplish; banner oblanceolate, 1 cm. long, emarginate; wings about 8 mm. long, the blade oblanceolate, with a large reflexed auricle; keel-petals about as long, the blade strongly arcuate near the rounded apex, with a small auricle; pod rounded ovoid, strongly cross-ribbed, glabrous, 4 mm. long, nearly as thick and broad, broadly obcordate in cross-section.

Type collected at San Luis Obispo, California, April 1886, *M. M. Miles* (herb. N. Y. Bot. Gard.).

12. *Hesperastragalus catalinensis* (Nutt.) Rydb. *Astragalus catalinensis* Nutt. Proc. Acad. Phila. 4: 9. 1848. This species has usually been regarded as the same as *Astragalus didymocarpus* H. & A., but differs in the narrow linear leaflets, deeply emarginate at the apex; and in the hairs of the pod, which are at least twice as long. The corolla is usually smaller.

CALIFORNIA: San Diego County, *Orcutt*, in 1884; *W. S. Brown*; *M. E. Jones 3152*; *Abrams 3517*.—LOWER CALIFORNIA: San Quentin Bay, *Palmer 614*.





# INDEX TO AMERICAN BOTANICAL LITERATURE

## 1923-1925

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The specific electrical conductivity of the leaf tissue fluids  
of phanerogamic epiphytes

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I. INTRODUCTORY

It has frequently been suggested that the specific electrical conductivity of organic fluids should furnish a satisfactory measure of their content of salts and acids. Practically, however, there are many difficulties to be overcome before such data determined in the course of physiological and ecological work can be fully interpreted. They have, nevertheless, much value when comparatively treated, and have been used in a number of investigations. It should be evident that in ecological work, for example, a knowledge of the electrical conductivity of the tissue fluids of halophytes should furnish information of much value when considered in comparison with that of the plant species of non-saline areas and of plants of transitional habitats. The value of such data will necessarily become greater as the number of series available for comparison becomes larger.

Among the problems which might seem of outstanding interest is that of the electrical conductivity of the tissue fluids of epiphytes as compared with those of terrestrial species.

In an earlier paper (*x*)<sup>1</sup> I have shown that the osmotic concentration of the tissue fluids of phanerogamic epiphytes is generally very much lower than that of terrestrial plants. Under the conditions available for the first investigation it was impracticable to determine physicochemical constants other than osmotic concentration. The primary purpose of the present paper is to give the results of a series of conductivity measurements made in 1923 on epiphytic flowering plants from the hammocks of Southern Florida.

<sup>1</sup> Reference is made by number (*italic*) to "Literature cited," p. 187.

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## II. METHODS

The methods are those employed in a series of investigations on the physicochemical properties of plant tissue fluids, and need not be discussed in detail other than to say that specific electrical conductivity was determined in a Freas cell, standardized against N/10 KCl, taken to have a conductivity of 0.01412 mho at 30°, after centrifugation and the determination of osmotic concentration by the cryoscopic method, on sap expressed after freezing the tissue in an ice-salt mixture.

## III. PRESENTATION AND ANALYSIS OF DATA

The values of osmotic concentration (in terms of both the corrected freezing point depression,  $\Delta$ , and in atmospheres, P) specific electrical conductivity in reciprocal ohms, K, and the ratio of conductivity to freezing point depression,  $K/\Delta$ , are given in the accompanying TABLE I.

The determinations on the practically leafless species of *Vanilla* were made on the fluids of the succulent stems. All others were based on the leaf tissue fluids.

The values of osmotic concentration are uniformly low, and serve to confirm the conclusions drawn from earlier investigations. The electrical conductivities are more variable in magnitude. Of the 22 values, 14 show a conductivity less than 0.010 reciprocal ohms, 6 show a conductivity less than 0.015 reciprocal ohms, and the remaining 2 are characterized by a conductivity of less than 0.020 reciprocal ohms.

Comparing these values among themselves, we may note that the lowest conductivities are those of the fluids from the succulent stems of the *Vanilla*. These are only about half as large as those found in the leaves of the other epiphytes, and are among the lowest conductivities of plant tissue fluids reported.

In general, the Bromeliaceae have a higher electrical conductivity than the Orchidaceae or Piperaceae. This may, however, be due in part to environmental factors. A number of the bromeliads were taken in mangrove swamps where there was doubtless an occasional opportunity for the absorption of salts from wind-borne spray, whereas the orchids came mainly from points more distant from salt waters.

In the case of *Tillandsia fasciculata*, C2366, in which  $K = 0.0180$ , was taken from a mangrove swamp on the shores of

the saline waters of Biscayne Bay, whereas C23155, in which  $K = 0.0100$  only, was taken from a mangrove swamp growing under essentially fresh water conditions in the Jupiter River. The only available conductivities for *T. utriculata* (C2364) and *T. aloifolia* (C2367), in which  $K = 0.0169$  and  $0.0115$  respectively, were based on plants growing in association with the *T. fasciculata* mentioned above (C2366) along the saline waters of Biscayne Bay.

The conductivities of these forms from the saline mangrove swamp are higher than those of *T. tenuifolia* (C2351) and *T. fasciculata* (C23155) from habitats more distant from the sea.

The 16 determinations on orchids are with three exceptions less than  $0.010$  reciprocal ohms. The highest value ( $K = 0.0117$  for C2365, *Encyclia tampense*) is based on materials taken in association with *T. fasciculata* (C2366), *T. utriculata* (C2364) and *T. aloifolia* (C2367) in the saline mangrove swamp.

The suggestion of a definite relationship between the salinity of the tissue fluids of the epiphytic vegetation is enough to justify the undertaking of more detailed comparisons between ecologically different habitats.

Turning now to a comparison of these conductivities with those which are available in published form for terrestrial plants, we may note that, while they are low as compared with the values found in saline habitats such as the *Kochia* association, the Shadscale association, the Greasewood-Shadscale association, and the more saline Grass-Flat and Salt-Flat associations of the Great Basin (3), they are lower than those found in mistletoes of the Southern Arizona region (11), and lower than that of *Cuscuta* parasitic on a halophyte, *Allenrolfea occidentalis* (2). They are also lower than those for the cottons cultivated on saline soils (10, 8), as might logically be expected from the high chloride (9) and sulphate (7) content of the latter.

The values are, however, not so small as might perhaps have been anticipated for plants growing without immediate contact with the soil. The higher constants are of the same general order of magnitude as many of the species of the Stansbury Mountains at higher elevations (3). They are of the same general order of magnitude as the conductivities of an extensive series of determinations on such trees as *Acer*, *Betula*, *Fagus*, *Juglans*, *Gleditsia*, *Platanus*, *Quercus*, *Robinia* and *Salix* as determined on samples collected at various levels (6) on Long Island.

TABLE I.

Species	Collection number	Date	Depression of freezing point $\Delta$	Osmotic concentration in atmospheres, P	Specific electrical conductivity K	Ratio, conductivity to depression K/ $\Delta$	Notes
<b>BROMELIACEAE</b>							
<i>Tillandsia tenuifolia</i> L.	C 2351	Jan. 17	.43	5.1	.0093	.0219	In Royal Palm Hammock
" <i>fasciculata</i> Sw.	C 2366	" 21	.61	7.3	.0180	.0297	In mangrove swamp, on shore of Biscayne Bay
" <i>utriculata</i> L.	C 23155	Feb. 8	.43	5.2	.0100	.0232	In mangrove swamp, on island in Jupiter River
" <i>albifolia</i> Hook.	C 2364	Jan. 21	.66	8.0	.0160	.0256	In mangrove swamp, on shore of Biscayne Bay
"	C 2367	" "	.55	6.7	.0115	.0207	In mangrove swamp, on shore of Biscayne Bay
<b>ORCHIDACEAE</b>							
<i>Vanilla</i>	C 23204	Mar. 1	.46	5.6	.0054	.0116	In dwarfed mangrove swamp, near Bay of Florida
"	C 23358	Apr. 13	.45	5.4	.0056	.0126	In dwarfed mangrove swamp, near Bay of Florida
<i>Macradenia lutescens</i> R. Br.	C 2341	Jan. 17	.43	5.2	.0068	.0158	In Royal Palm Hammock
<i>Oncidium undulatum</i> (Sw.) Salisb.	C 23353	Apr. 14	.34	4.1	.0079	.0229	In <i>Conocarbus</i> swamp, near Bay of Florida
<i>Epidendrum cochleatum</i> L.	C 2342	Jan. 17	.42	5.1	.0080	.0190	In Royal Palm Hammock
"	C 23125	Feb. 2	.45	5.4	.0111	.0250	In a Pineland Hammock
"	C 23148	" 8	.42	5.2	.0090	.0210	In wooded swamp, at Headwaters of Jupiter River
"	C 23186	Mar. 1	.46	5.5	.0105	.0228	In <i>Conocarbus</i> swamp near Bay of Florida
<i>Spatholiger rigidus</i> (Jacq.) Small	C 2356	Jan. 17	.38	4.6	.0093	.0244	In Royal Palm Hammock
"	C 23147	Feb. 8	.32	3.9	.0070	.0219	In wooded swamp, Headwaters of Jupiter River
"	C 23302	Apr. 4	.31	3.7	.0076	.0250	In wooded swamp, Headwaters of Jupiter River
<i>Encyclia tampense</i> (Lindl.) Small	C 23107	Jan. 24	.45	5.4	.0092	.0206	In mangrove swamp, on island in Jupiter River
"	C 2365	" 21	.64	7.7	.0117	.0183	In mangrove swamp, on shore of Biscayne Bay
"	C 23149	Feb. 8	.65	7.8	.0091	.0141	In wooded swamp, Headwaters of Jupiter River
<i>Auliza nocturna</i> (L.) Small	C 23183	Mar. 1	.51	6.2	.0090	.0177	In mangrove swamp, near Bay of Florida
"	C 23350	Apr. 13	.38	4.6	.0096	.0249	In mangrove swamp, near Bay of Florida
<b>PIPERACEAE</b>							
<i>Peperomia obtusifolia</i> (L.) Dier.	C 22038	Dec. 14	.46	5.5	.0126	.0273	In Royal Palm Hammock



Remembering that the tissue fluids of ligneous and herbaceous plants are differentiated with respect to their electrical conductivity (4), we may properly compare these with a series of determinations on herbaceous plants (5) from Long Island.<sup>2</sup> We may note that the average value of K for a series of 162 species of herbs is 0.014308. Examination of the individual constants shows that a number of the species have tissue fluids of as low conductivity as that found in the epiphytes.

#### IV. SUMMARY

The osmotic concentration and specific electrical conductivity of the tissue fluids of a number of phanerogamic epiphytes from the subtropical habitats of southern Florida have been determined.

The low values of osmotic concentration are in excellent agreement with earlier series from Florida and from Jamaica, British West Indies.

The specific electrical conductivities are variable. This is apparently due to the fact that some of the species were under the influence of the greater salinity of strand conditions.

While the conductivities are far lower than those of terrestrial species in saline regions, they are of the same general order of magnitude as the lower values of herbaceous forms in non-saline regions. It is clear, therefore, that the tissue fluids of epiphytic plants are by no means poor in conducting solutes.

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## The nature and cause of secondary sexual states with special reference to *Typha*<sup>1</sup>

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Any sexual condition developed in the individual usually exhibits great stability, and when sex reversal is induced we are very greatly impressed with the peculiarity of the phenomenon. Yet, if a man were acquainted only with hemp grown in a nine-hour daylight period and then were suddenly transported to a region where it was raised in a fifteen-hour daylight period, he would be just as much surprised at the apparent change from a condition of sexual instability to the very fixed condition and segregation of maleness and femaleness exhibited in the new environment.

Because of the usual stability of unisexual individuals, there has been a more or less tacit assumption among biologists that the persistency of a given sexual state must be an adequate basis for a differential factorial explanation of that duality of form and function which we call male and female. The apparent predisposition, either in the young or mature individual, toward one sex or the other, the seeming fixity commonly present and the frequent inability of the experimenter, hitherto, to produce reversals of the sexual state at will, have appeared to many to be conclusive indications of a specific and particular condition of allelomorphic hereditary factors, either of a simple or multiple nature.

But just as unisexuality has its antecedent in hermaphroditism, so has persistency of structure and function, as postulated in our theories of heredity, its counterpart in the persistency of structure and function of differentiated tissues and organs coming from a common source and whose complement of hereditary potentialities are known to be absolutely the same. It is quite well known that we have made less progress in dedifferentiation, in undoing the results of differentiation of highly specialized

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organs, than in the changing of sexual states or inducing sex reversals. It is this view, that persistency of sexual states is due to differentiation processes of the same nature as other differentiation processes and not to differential hereditary factor systems, that is the text of the present discussion.

We are all aware of the stability in the development of roots and shoots. In a very early stage of the embryo an incipient shoot bud and an incipient root bud are organized and in the simpler cases the root has not only a very profound difference of form, structure, and chemical content from the shoot but the two systems have an entirely opposite reaction to the physical environment, the root growing and turning in the direction of gravity and the shoot against it. This property, continuing, places the two parts into two extreme environments, soil and darkness, etc., on the one hand and air and daylight on the other. Now these two conditions are persistent and stable, the root producing more root with the same characteristics and properties and the shoot producing its same characteristics and properties. What is the cause of this persistency of character and action which continues the remarkable dimorphism? The answer is obvious. It does not result from a difference in potentiality of the cells, in other words a difference of heredity in the two parts, but is due entirely to the course of differentiation starting in the embryo and not changing in the meantime, nor do we expect it to change unless we produce a proper alteration in the environment. By such a change of environment the root in many species immediately begins to produce the complete and normal shoot and the shoot in the same way begins to produce root. Not only are morphological expressions thus stable but the same conditions of fixity are to be seen in differentiated functional systems. Certain organs are differentiated and continue to produce the same materials and reactions so long as the plant or animal lives. The liver continues to secrete bile, the base of the fingernail keeps on producing more fingernail, not because the two sets of cells have different potentialities—they have received the same common heredity—but because of the fact that they have become differentiated in these functions during the early ontogenetic development through the guidance of physiological and growth gradients.

The process of differentiation may also acquire a stability

which persists only for a time and suddenly changes to another type. For example, the persistency of dwarf branch and long branch in *Ginkgo* may be considered. For many years, through cold and heat, dry season and wet season, a bud will continue to produce a dwarf branch, characterized by the absence of internodes and functional axillary buds, yet suddenly through some internal cause it will begin to develop as a long shoot with long internodes and well developed axillary buds. Sooner or later the factors inducing dwarf-branch characters will again come into play. Some dwarf branches, however, may continue their development for years and finally die without a change. We can then say that this special bud was "permanently determined" since it showed a constancy during its entire ontogeny, yet we know that a little manipulation would have brought about a change to the long branch condition. Just so, we say that a plant is "permanently male or female" if it shows no sex reversal before its death.

It has become evident from various experiments on the higher plants that there is no direct relation between unisexuality or monosporangiateness of the individual and fixity of the sexual state on the one hand, and hermaphroditism or bisporangiateness of the individual and modifiability on the other; nor is there any relation between extreme sexual dimorphism and stability. As shown by experiments on the extremely dimorphic hemp, sex reversal is often more easily induced than in species much less dimorphic. The progression of the development of sexual states with the accompanying morphological expression normally follows in an almost invariable order in the developing floral axis of bisporangiate flowers, as in a *Magnolia* where a broad zone of stamens is developed first, followed, through a reversal of the sexual state, by a broad zone of carpels. Bisporangiate species are then also decidedly fixed as to the sequence of sexual expressions and persistency of sexual states within the limits of their ontogeny. However, experiments on monocious species especially are needed and promise important results, judging from what may be learned by direct observations and from the experiments so far conducted on the partly monocious *Arisaema Dracontium*, in which monocious individuals can be changed to the pure staminate condition and vice versa. In any event, a most favorable starting point for the study of the origin and

stability of sexual states in the ontogenetic cycle can be found in those types of monocious plants in which first one sexual state is established in a determinate inflorescence bud to be followed later through sex reversal to the opposite state, a very uniform sequence of expression being present because of a terminating physiological gradient. In such cases the sexual states and expressions can be followed out more clearly because it is known beforehand that they all come from a common hereditary potentiality. In this way we can discover whether there is any fundamental difference in the nature of sex determination between those organisms which are unisexual and so may have a differential heredity and those which are bisexual or hermaphrodite.

The two species of *Typha* were selected for special study in this respect. Although only field observations have been made so far, they nevertheless appear to offer very important evidence. In both species the carpellate inflorescence is normally below and the staminate above, both types of flowers being greatly reduced and developed in great numbers directly from the inflorescence axis which is a continuation of the aerial leafy shoot. The two species, *T. latifolia* and *T. angustifolia*, are essentially alike in their sexual expression, both normal and abnormal, except that the first usually has the carpellate and staminate parts in close proximity or contiguous, while the latter usually has a short stem between the two. This distinction, however, often breaks down; *T. latifolia* may have a long stem between the two parts and in *T. angustifolia* the two may be contiguous. The carpellate and staminate zones of the inflorescence are usually about equal in length. The normal development of the axis is, therefore, for the bud, as it approaches determinate growth, to pass to the female condition and to develop a wide zone of carpellate flowers and then, after passing through a neutral condition for a greater or lesser period of time, to reverse completely to the male condition which continues until growth is ended. There are, however, variations from this general type. In some cases a tiny patch or zone of carpellate flowers is first produced, followed by a long neutral stem which finally ends in a staminate inflorescence. Quite frequently stalks develop with a purely staminate inflorescence, no femaleness whatever being expressed. When reproduction begins, the bud passes over to the male state and continues thus to the end of development. This condition has been

observed in Maine and Ohio and especially in northern Michigan, Wisconsin and Minnesota. These purely staminate shoots arise side by side with the normal monocious shoots with carpellate flowers below and staminate above. In these cases of purely staminate inflorescences, the functional gradient is the same in the growing bud at the beginning of floral development as it is in the normal type of inflorescence only after passing through the female condition and then back through neutral to the staminate expression.

Although there is sometimes a great difference in the comparative lengths of the carpellate and staminate parts, and as intimated all gradations can be found to the complete absence of femaleness, so far no case has been observed in which the entire inflorescence was carpellate. A few specimens have been collected in which the lower carpellate part was very long and the upper staminate part very short. Sometimes the female state continues until a foot or more of carpellate inflorescence has been developed. It would probably require a very extreme and extraordinary condition to keep the female state intact until the end of determination. However, it is possible that such a condition may occasionally arise in view of certain extraordinary facts presented below.

In some cases inflorescences are developed in which a bud is thrown into the male state on one side and the female state on the other, or several alternating segments may show alternating sexual states. In such cases, since the sexual states are persistent for some time, vertical strips or bands of carpellate and staminate flowers will be produced. These inflorescences then appear like sectorial chimeras; but while chimeras are produced by the peculiar arrangements of different species of cells with different heredities, these chimera-like inflorescences are developed from cells with a common heredity but with different, persistent sexual states. However, at the proper level the cells in the female condition will be reversed to the male state and the upper part of the inflorescence thus becomes normally staminate. In some cases only a very small group of cells will be in the male state at first and these will give rise to a very narrow strip of staminate flowers in the carpellate inflorescence. In still other cases, when flower development begins, a minute group of cells of the developing inflorescence bud will be thrown into the male state and begin to

produce staminate flowers, while the greater part is in the female state and producing carpellate flowers, and the area of the bud in which the cells are in the male state will gradually increase until the entire bud is finally in the male state. In other words, there is a gradual spreading of the area of sex reversal. In such an inflorescence the lower part will be carpellate and the upper staminate with a long diagonal plane separating the contiguous female and male parts.

In inflorescences with three internodes or divisions, it frequently happens that the lowest internode is entirely carpellate, the middle node is carpellate on one side and staminate on the other, the one vertical band of flowers being wide and the other narrow or both being of about the same width, while the uppermost internode is entirely staminate.

In all these cases the progress and persistency of expression is from carpellate to staminate, and when the staminate condition is attained it persists to the end of determination. There is a definite progression of events just as in morphological differentiations where sex is not involved. But just as sex reversal is possible and easily induced in various dicocious species, and as the differentiation progression or the differentiated cells can be redifferentiated (as is the case in hemp, where three or four rejuvenations are possible and three or four differentiation cycles, of the same nature as the first one that had its inception in the seed, may be passed through) so it must be possible for the physiological states and gradients in the *Typha* to vary in respect to sexual differentiations and so induce reversals of the sexual states other than those that the normal cycle indicates and thus give reversed arrangements in the succession of carpellate and staminate flowers. The degree or extent of such changed expressions will depend on the specific hereditary constitution and the fitness and intensity of the environmental conditions which can bring such changes about. So far not many aberrant examples of this nature have been found. No case has been seen in which the inflorescence was staminate at the base and carpellate above. Whenever male determination took place at the base of the inflorescence it continued to the end of development. One type of reversal from maleness to femaleness is, however, rather common, and was first called to my attention by a former Chinese student, Mr. C. K. Kao. An inflorescence with a normal carpel-



late zone below and a staminate zone above may reverse at the very tip and develop carpels again. Commonly, these carpellate flowers, although they have stigmas, have imperfect ovularies. A few cases were found, however, where the carpellate development covered a half inch or more of the tip and appeared to have some perfect or nearly perfect carpels. More interesting were three shoots of *Typha latifolia*, found in Northern Michigan, which had the normal, basal carpellate zone and the normal reversal to the staminate condition near the middle of the inflorescence and then, after about an inch of growth, had reversals to femaleness, developing patches of normal carpellate flowers again. In one of these a neutral area began to appear about an inch above the base of the staminate zone on one side, which soon changed to a normal female tissue with normal carpels. The neutral area was covered with papillae which gradually merged through imperfect to perfect carpellate flowers. The other two inflorescences each had two oblong patches of perfectly developed carpellate flowers on opposite sides within the staminate zone, beginning at somewhat different levels but both terminating rather abruptly through a reversal to the staminate condition again. Here then, beginning at the base of vertical bands of the inflorescence, certain groups of cells of the inflorescence bud reversed their sexual states in their cell lineage from neutral, to female, to male, to female, to male; or in the first example cited from neutral to female, to male, to neutral, to female, to male. Although we have in this case temporary stability at each stage, the group of cells in question nevertheless passed through three sex reversals rather than one, as is the normal condition. There can be no question but that with proper investigations such changes could be produced at will in the monocious *Typha* as readily as they have been brought about in the diecious *Cannabis* and other diecious species.

In the observations just given, we have the very same phenomena of stability and instability of sexual states in a monocious plant as those which have been produced in the diecious *Cannabis sativa* without allosomes, and the diecious *Lupulus japonicus* with allosomes; in both of which there is a normal condition of stability under the usual environment but instability accompanied by abundant sex reversal in unusual environments, either during the original ontogenetic cycle of the plant or, in cases of repeated rejuvenations, in a subsequent cycle of development.

Now it must be emphasized that in all such cases which involve reversals and rereversals of sexual states and expressions in monocious species, we know that we are not dealing with different hereditary constitutions or changes in such constitutions but merely with functional states and physiological gradients and with plus (+) and minus (—) states, which are responsible for the activity and latency, or for a peculiar mode of control or reaction, of the hereditary factors present in the complex. The persistency of a given sexual state is then of the same nature, and caused in the same way as the persistency of any ordinary vegetative differentiation, and the change from one sexual state to the other is brought about in the same way as a change in vegetative expression is brought about, through the operation of the usual ontogenetic gradients, or in case of rejuvenation by new gradients induced by environment.

From the experiments on reversals in dieocious species and observations on the sharply bounded limits of male and female tissues, it appears that in plants there are no hormones produced which are readily diffusible from one cell to the other. If such substances are produced in relation to the sexual conditions, they must either be developed so constantly and in such quantity as to be able to neutralize any material coming through from a region of the opposite sexual condition, or else the peculiar character of the plant cell wall offers a very decisive barrier to the passage of such substances. It is now known that cell walls of the higher plants usually contain layers of pectose as well as cellulose, and pectic walls are known to be differentially permeable to certain substances. In some of the higher animals such hormones as are produced in the sex glands appear to circulate through the body and to be readily diffusible through the animal cell wall. Thus, in many animals at least, not only the sexual state but the differentiated tissues become unstable and reverse their sex whenever the opposite hormone is introduced into the system in suitable quantity. The transformation is sometimes so extreme that it is difficult to determine the original sexual state of the animal unless the allosome condition were studied which presumably should not undergo a transformation. But hormones produced by the sex glands are not the primary cause of sexual states. They are the results of such states but they are of such a nature, nevertheless, that their presence is a

proper environment to produce sexual expression, determination, or reversal.

It is important to remember that just as sex reversals are brought about in monocious species in apparently the same external environments, so reciprocal reversals—male to female and female to male—occur and are induced in exactly the same external environments, so far as one can see, in diecious plants when they are growing side by side, as is the case with hemp and Japanese hop. But the physiological gradients and conditions in the different individuals and in different parts of the same plant are necessarily different at practically all times. So if we originally get equal numbers of males and females of the common hemp in a common environment, this is no more an argument in itself for the hypothesis that the result is due to a difference of heredities than the fact that through the influence of a common environment we get reciprocal sex reversals; for this in itself shows that the two individuals were exactly similar in so far as sex potentialities are concerned.

We must not confuse sexual characters with sexuality itself or the sexual state. For sexuality is present in the lower forms without any expression of it in morphological characters. It is purely a functional state which manifests itself in isogamous gametes after their development is complete. Now if the time of origin of a sexual state is thrown back into the beginning of gametogenesis, there can be a difference in morphological expression. In the lowest heterogamous gametes this difference in character manifests itself then mainly as a difference in size. With the progression of evolution the hereditary complex of factors which may be influenced either by a primary or secondary sexual state in the cell becomes greater and results in greater dimorphisms.

Every unit sexual character is the result of the activity of a unit factor. But the expression of this factor, whether in relation to its activity or latency or the peculiar nature of the character itself, depends on the sexual state present at the time. And this state may be very fixed or very evanescent. The factors responsible for sex-limited characters will be segregated in the ordinary Mendelian fashion or according to any type of segregation and union taking place in the chromosome complement. But the determination of sexual states in itself is not a matter of

the presence or absence of a specific set of genes, since we know that the cell can change from one condition to another without a change in chromosome content. Now we do not at all deny that there are segregative factors that will change the functional activity of an organism or modify its gradient so that sexual changes of expression are advanced or retarded, up or down the developing axis, but the direct action on the sexual state is exactly the same as when sexual states are influenced through the environment. The environment produces a change in the physiological state which causes one or the other sexual state to be developed or reversed. It is on this account that environment may change the course of the expected sexual expression. The environment is not changing the nature of the fundamental potentialities responsible for a given sexual character.

Emerson has obtained mutants of Indian corn, which under ordinary conditions are entirely female, the terminal as well as the lateral inflorescence bearing carpellate flowers only, and hereditary factors have been found which when present in any constitution produce this condition. Now at first glance it might appear that here we have a true sex factor. But, as intimated, this factor may have nothing to do directly with sex but may be such as to affect the metabolism of the cell in a certain way in a given environment and this functional condition results in the development of a female state both on the side-ear branch and on the erect branch which terminates the growth of the shoot. From what we know of such conditions of sexuality, it appears probable that a special environment may be found which would act on the developing plant in such a way that a new gradient would be established in the terminal shoot which would not allow femaleness to develop in it or else would bring the physiological state to such a condition that reversal to maleness would come about at the base of the tassel or before the growth of the terminal inflorescence was ended. There are mutants of Indian corn in which the terminal stalk does develop femaleness and is partly modified to resemble the shank of an ear with partially developed husks and sometimes with little axillary ears, but the female state nevertheless disappears and the stalk ends in a pure staminate tassel or with carpellate structures developed only at the very base. If the ordinary types of Indian corn, which are monocious in the usual seasonal environ-

ment, are planted in the greenhouse during the short daylight period of winter, many individuals will show only female expression, producing carpellate inflorescences both at the side and at the tip or more commonly at the tip only. This is plainly due not to a lack of potentiality to produce the male state nor to a lack of any of the hereditary factors required for the expression of typical male characters but to the establishment of a new gradient of growth and function which causes reproduction to begin much earlier in the plant than when it is grown in the usual environment. The only difference I can see between the first and the third cases is that the first has one or more vegetative factors which have an influence on the functional activity, causing a different reaction in the same environment in respect to sex. This gradient and consequent reaction will not be the same in a different environment, and, since it seems quite certain that the carpellate corn plants have all the hereditary potentialities for male characters, the variety will show maleness whenever a suitable environment is found.

Now if one is still inclined to think that such factors should be called sex factors in the ordinary sense of the term, then the matter becomes merely a case of confusion of terminology, for it is evident that up to the present time the term gene or hereditary factor has had no such meaning. When we say we have a pair of allelomorphic factors for red and white pericarp, no breeding experiments have ever shown that after you have segregated the red and white you can still get the opposite characters out of the segregates as we can do in the case of male and female. The genes responsible for the carpellate Indian corn variety can be segregated out like any other Mendelian factor. The sex genes supposed to be responsible for sexual states are not so eliminated, for when the one is supposed to be segregated out of the system, as for example in the very popular "homozygous heterozygous formula," we can still take the supposed homozygous individual, which is presumed to have the homomorphic allosomes or the homozygous sex factors, and in many cases bring out the opposite sexual state. What was labelled "homo" has by some mysterious magic become "hetero."

That differentiation results in varying degrees of fixity is plainly shown by experiment. In the case of sexual differentiation, Mrs. Wuist Brown was able to induce changes of female

gametophytes of the ostrich fern up to 90 per cent, but the male gametophytes were much more fixed. With the treatment given, only 5 per cent showed reversal from maleness to femaleness. This agrees with the usual expectation since it is generally recognized that the male condition is a more extreme condition, when compared with the neutral vegetative state, than the female. In the diploid, highly dimorphic diecious hemp, however, no such difference in physiological stability is to be observed. The staminate plants show reversal to femaleness even more readily than the carpellate plants do to maleness, and usually much more promptly, when subject to exactly the same environments. We cannot then judge the degree of physiological differentiation or stability by the degree of morphological difference visible.

Czaja, in his experiments on regeneration from archegonial and antheridial tissues taken from the hermaphroditic gametophytes of homosporous ferns, found that the protonemata from archegonia can be made to develop both sexes and those from antheridia also, but it was quite difficult to produce the change in the plants which had originated from the male tissue. In these cases then, it is plain that the greater fixity present in the haploid male condition is not due to a difference in hereditary potentialities, since both have come through vegetative growth from a common haploid complement of chromosomes, but is dependent entirely on the differential processes of differentiation going on in the presence of the one or the other sexual state.

In species of *Oedogonium* with dwarf males it is known that the individual is haploid. The individual instead of producing male and female gametes, as in normal *Oedogonia*, produces eggs and the so-called androspores which are to be considered merely as parthenogenetic sperms. These are in the primary male state to a certain degree, at least, and are attracted by the eggs but instead of fertilizing the eggs they usually settle down on the base of the oogonium or on the cell below it and develop into a new filament, the dwarf male, which finally gives rise to functional spermatozoids. The dwarf male develops its peculiar morphology and persists in the male state not because it has a different complement of chromosomes or a different hereditary complex but entirely because of the fact that it is differentiated in a male state.

We well know that in the heterosporous plants, namely in all

higher plants, the sexual state which has been determined in the diploid sporophyte, or any part of it, is not changed in the passage over to the gametophyte but persists through the reduction, all four spores of the reduction showing the same sexual state. This is true whether the plant has bisporangiate flowers, whether it is monocious or diecious, whether it has allosomes or not. That there is occasionally a reversal in the sexual condition just before or after reduction is shown in such cases as reported by Chamberlain for *Salix*, where he found pollen grains inside of the ovules.

In case allosomes are present, if the carpellate plant is homozygous for allosomes and the staminate plant is heterozygous, the reduction division, although it segregates the allosomes with any differential heredity that they may possess, does not influence the sexual state, but the same secondary sexual condition remains in the cells with each kind of allosomes, all four giving rise to microspores with secondary male characters and these in turn all develop male gametophytes which finally produce male gametes with primary male states. Now if these allosomes were secondary sex producers in the diploid sporophytes, allosome *B*, of the heterozygous, staminate Japanese hop plant, being dominant over allosome *A*, might be expected to continue maleness when alone, but what is it then that continues maleness in the microspore and male gametophyte when allosome *A* is alone, the assumed, activating cause of maleness, the dominant allosome *B* being absent? In the carpellate plant the homozygous allosomes *AA* are assumed to determine the femaleness, and after the reduction division the female state is continued in all the spores produced and their following gametophytes. Why do the cells with allosome *A* produce female gametophytes in the one case and male gametophytes in the other if they are the cause of sex determination? In the diploid carpellate plants the homozygous allosomes *AA* are assumed to determine the femaleness and after the reduction division the female state is continued in all the subsequent haploid cells. The microspores, which have the single allosome *A*, produce male gametophytes exactly the same as those with allosome *B*. The allosome *A* is absolutely impotent and does not influence either the primary or secondary sexual states which have been established before the reduction division. If the conditions are the reverse in some higher plants,

the carpellate individual having the heteromorphic pair of allosomes, the results will still be the same except that in this case the allosome *A* would have no ability to change the sex to maleness in the gametophyte coming from the megaspore. It must be recalled again that in such plants with allosomes like the Japanese hop, the carpellate plant homozygous for *A* can easily be reversed to the male condition and the staminate plant with the heterozygous *AB* can easily be reversed to the female condition.

In those higher, homosporous, vascular plants in which unisexual gametophytes are developed, the determination presumably takes place in the spores. Now these haploid gametophytes, as stated above, can also be made to reverse their sex, the male to the female and the female to the male, so it is self-evident that both haploid complements of chromosomes contain the potentialities for both sexes, and determination could not have been brought about in the first place because of any sex-determining factorial difference, nor could it have been reversed later because of a factorial difference. It is evident that the process of reversal must be ultimately the same and dependent on the same conditions as the original sex determination.

In the higher animals with a simple diploid life cycle, the conditions are the same as in the higher plants. The secondary sexual state present in the individual is passed on to the cells after reduction, whether only three or four are produced or many, as is the case in a few species, and ends in the same primary sexual state. The segregation of chromosomes and hereditary factors, even when allosomes are present, has no influence on the secondary and primary sexual states of the generation of haploid cells. If the male has the heteromorphic allosomes, all the four cells coming from the spermatocyte through reduction, both those with allosome *A* and those with allosome *B*, develop the primary male state, the allosomes showing a complete impotence to effect either the secondary or primary sexual states or the morphology that goes with them. In the female all the cells which develop as eggs have allosome *A* and continue the female condition not because of the presence of this chromosome but because the sexual state handed over from the diploid female individual has in the meantime not been changed. If the female individual has the heteromorphic allosomes, some of the reduction cells will



have the allosome *A* and some will have *B*, but all that develop will continue the female state handed over from the diploid female individual, the allosomes are as impotent to influence or change the female condition as they are in changing the male state.

It is positively known from the work of Blakeslee, Belling, and others that a haploid complement of chromosomes works in practically the same way as a diploid set. In the haploid sporophytes of *Datura* the sporophytic characters appear practically normal, having the usual *Datura* characters and developing the usual male and female characters in the flowers. Furthermore, we know that the triploid condition does not change the character essentially. The cause of endosperm development from a triple-fusion cell is not at all due to the triple complement of chromosomes but must be due to the differentiation and functional conditions of the cells involved. The triploid condition may result in a sporophyte as well as the diploid or haploid condition.

So there is no opportunity to attempt to explain away by ingenious, imaginary hypotheses and fairy tales the known impotency of the allosomes to produce sexual changes and determinations in the gametophytes by the plea that the allosomes, although impotent in the haploid condition, are powerfully potent in the diploid condition. As shown in the discussion above, they are certainly impotent in the diploid condition when it comes to a contest with external factors.

It seems that in nearly all cases the reduction division cells remain in the secondary sexual condition which was developed in the tissue from which the reduction cells arise or else, as in some lower forms, they are in a neutral condition, as in *Oedogonium* and other genera. So far as I am aware there is only one case known in the whole realm of living things where differential allosomes are associated with definite sexual states in the haploid condition, namely in *Sphaerocarpus* as worked out for the most part by Allen. And here again we have the same puzzle, only reversed, as in the higher plants and animals. If we say the allosomes of *Sphaerocarpus* are sex-producing, to say nothing of sex-determination, their potency vanishes so soon as we get them in a diploid condition, for a completely non-sexual sporophyte is the result of their association in fertilization. The

generally assumed dominance of one of the heteromorphic allosomes on which the entire homozygous heterozygous hypothesis is established, is singularly lacking. Yet in recent times investigators who have been dealing with the supposed allosomes in higher plants, where they are absolutely impotent in producing any sexual changes whatever in the gametophytes, have not hesitated to consider their discoveries as being of the same nature as those in *Sphaerocarpus*. How are we to explain the differential association of sex and allosomes in *Sphaerocarpus*? I answer, in the same way as we do in other cases. The allosomes probably contain differential functional factors which have enough potency to set up a proper physiological condition in the given environment, which throws the sex one way or the other. To get a different association of the sexual state and the allosome it will be necessary to find a proper environment in which to develop the individual, and then, as in the case of a reversed hen or a reversed Japanese hop plant, the sexual state of the individual and the allosome condition will be just the opposite from what they are in the normal.

Sexuality manifests itself in several progressive or orthogenetic stages, in organisms in general, which may be summarized as follows:

First, in the lowest condition, it arises from time to time in the cell lineage of the lowest sexual organisms as a functional plus (+) or minus (—) condition of morphologically quite similar gametes, giving them the property of attraction and fusion. In this stage it is purely a physiological and chemical manifestation.

In the second stage of evolution, sexuality shows itself in the early developmental stage of the gametes, giving them a primary sexual dimorphism of the usual heterogamous type, common to all the higher plants and animals, in addition to the primary functional property of attraction and fusion. This evolution of heterogamy is represented by a number of progressive stages. In the lowest heterogamous gametes, the only dimorphism exhibited is one of a difference in size. The highest in the series show not only a very great difference in size but also a decided difference in character and function, represented by the large stationary egg and the small motile sperm.

The third stage is represented by the appearance of secondary sexual states in cells and tissues outside of those destined to

become gametes and giving rise to secondary sexual characters, in the lowest condition involving only the cells immediately contiguous to the gamete producing cells or cells in the immediate vicinity of the gamete producing cells. In the higher types considerable areas of the body are differentiated as male or female tissues. This type of sexual expression includes all hermaphroditic animals, all hermaphroditic diploid plants with a simple life cycle, all hermaphroditic haploid plants with a simple sexual cycle, and all the plants with the lower type of alternation of generations as well as all those with the higher antithetic alternation of generations which have hermaphroditic gametophytes.

The fourth stage of advancement in sexual evolution is represented by all those organisms on the one hand with a simple life cycle with diploid or haploid individuals, in which the sex is determined in the spore or in the fertilized egg and consequently in which secondary sexual dimorphism may appear in the entire individual, the individual being either male or female; and is also represented on the other hand by all organisms with haploid unisexual gametophytes and homosporous sporophytes. Examples of this stage of sexual evolution are unisexual fucoids, all the unisexual animals up to man, and all the bryophytes and homosporous pteridophytes with unisexual gametophytes.

The fifth stage is represented by those organisms with a typical antithetic alternation of generations in which the time of secondary sex determination is thrown back into the vegetative phase of the sporophyte, the sporophytic individual being bisporangiate and the gametophytes unisexual. The bisporangiateness of the sporophyte also is evolved in a series of stages in which the maleness and femaleness of the sexually differentiated regions is determined in earlier and earlier stages of the ontogeny. In the lowest stage maleness and femaleness are determined side by side in neighboring sporangia; in the more extreme evolutionary stages entire branches of a monocious individual are determined as male or female. In this category come the vast majority of species of heterosporous vascular plants.

The sixth and last general stage of sexual evolution is represented by the heterosporous plants with unisexual gametophytes and diecious sporophytes. In this condition there are four distinct individuals in the life cycle which manifest secondary

sexual states, two dimorphic gametes with primary sexual states, and two very dimorphic nonsexual spores with secondary sexual states. The sex is determined in the zygote or fertilized egg and this determination remains throughout the alternation life cycle until the following zygote, at which stage it is determined anew. Sex reversal apparently may readily take place anywhere in the body of the sporophyte or in its sporocytes, but probably is practically impossible in the gametophyte unless it were possible to develop it independently outside of its normal environment, the sporangium.

But these last two types of sexual organisms each have a still more complicated phase in their angiospermous members, in that in addition to the egg, the female gametophyte develops two or more special cells, with primary sexual states, which unite with each other and with the second sperm about the time of the union of the egg and sperm; and from this triple or multiple cell fusion a special nourishing generation arises, the so-called xenophyte or endosperm. Now each stage of sexual evolution shows all the characteristics and usually also the degree and extent of sexualization of all the stages antecedent to it, so that at each stage there is a progressive addition to what was evolved previously.

Why should we think that the facts of sexual duality and determination need a factorial explanation when no such assumption has ever explained the most patent phenomena in relation to sex? Why do theorists not concern themselves with a factorial explanation for the cause and determination of the far greater dimorphism exhibited between sporophyte and gametophyte? Why is no factorial explanation forth-coming for the cause of the dimorphic expression of the sterile and fertile shoots of such plants as *Equisetum arvense*? We do not find any factorial explanations of the fact that in certain ferns highly dimorphic foliage leaves and sporophylls develop. We do not hear discussions concerning the probable factorial cause of root and shoot development in an ordinary plant. We do not read highly entertaining dissertations on the hereditary cause of the fruitfulness in a tree one year and of its almost complete sterility or unfruitfulness another. Then why should we attempt to find such an explanation for the dual expression of sexuality? But some one will say these cases do not involve individuals but only parts of

individuals. Well, so are the lower plants and animals practically all hermaphrodites and the higher sporophytes very largely bisporangiate individuals. As stated, at the beginning of the evolutionary series, sexuality manifests itself purely as a functional state of the cell which may come and go, be one sexual state in a cell lineage at one time and another state at another time; and so it has remained up to the highest organisms. In the evolutionary progression the cell has acquired a greater and greater aggregate of potentialities or hereditary factors, if you please, which can be activated, influenced, or inhibited by the presence of the one sexual state or the other, but the fundamental reality of sexuality has remained the same, a reversible physiological condition of the protoplasm. In the evolution of chromosomes the fact of sexuality has made possible the development of allosome sets of chromosomes which commonly show a definite association with one sex or the other in many unisexual and monosporangiate species, but unisexuality is just as prevalent in organisms where no such association of allosomes is indicated as in the unisexual gametophytes of all the higher plants. Allosomes are merely indicators of sex in certain groups of organisms. They follow the sex rather than determine it. They do not appear to be directly responsible in any case—although it is possible that they might be indirectly concerned—for any specific sex determination; nor are they responsible for the fixity of the sexual states when present, since it is absolutely established that sex reversal takes place in their presence. There are allosome-linked hereditary factors as there are autosome-linked factors and there are sex-limited or sex-influenced factors in both allosomes and autosomes as well as factors which are not sex-limited. Sex-limitation may be accomplished in single dose but not in double dose, or even a double dosage may be inhibited by a given sexual state and these conditions apply to the hereditary potentialities of both allosomes and autosomes. But, properly speaking, there are no sex chromosomes and especially is it true that there are no "sex-linked" factors. Such terminology leads only to confusion of ideas. Sexuality is a potentiality of all organisms or cells except the very lowest. This potentiality probably has its basis in some structural condition of the protoplasm. This potentiality is responsible for the development of sexual states, which may be male, female or neutral. Any cell or any in-

dividual not differentiated beyond the possibility of regeneration or rejuvenation has the potentiality to pass into any of these states. Depending on the sexual state present, various specific substances are produced in the cell and these as well as the state itself influence hereditary expression; that is, they activate, inhibit, or modify hereditary expression, or the hereditary factors. The cause of the establishment of any sexual state in any degree of intensity or stability is to be sought in the physiological activity of the cell.

Finally then, the persistency of sexual states is due to differentiation processes which proceed in the same manner when sexual differentiations take place, whether morphological or physiological, as when nonsexual differentiations are accomplished. Or as Stout has stated the matter: "The morphological differentiations of sex are fundamentally an extension of the phenomena of somatic differentiation."

COLUMBUS, OHIO.

## Additions to the genus *Lycianthes* Dunal

H. H. RUSBY

The reinstatement and extension of the genus *Lycianthes* by Dr. George Bitter (Abh. Nat. Ver. Brem. 24: 292-520. 1919.) is an important contribution to the literature of the family *Solanaceae*, especially in view of the critical and accurate manner in which the work has been performed. Every botanist who has done much work in this family must have been dissatisfied, at times, with the best disposition that he could make of his species. The difficulty is inherent in the family itself, which seems incapable of any perfect natural arrangement based on structural characters. *Atropa* and *Scopolia*, related naturally, are separated by the circumscissile pod of the one and the berry of the other; yet we cannot overlook the fact that *Scopolia* and *Hyoscyamus* possess equally natural affinities. *Solanum*, at one end of the family, has species with the saccate bilabiate corolla which characterizes genera at the other end. *Capsicum* and *Bassovia* appear perfectly dissociated as natural groups, yet their structural distinctions are most slender, and at times fail. The genus *Lycianthes* itself is not wholly satisfactory as a natural group. Its basic characteristic, the possession of 10 calyx-teeth, is not absolutely constant, and its wide and diverse distribution is evidence against its naturalness. Nevertheless, *Lycianthes* affords us the means for eliminating many glaring inconsistencies that have offended all special students of the family. Made up, as it is by Bitter, of 5 species from *Capsicum*, 7 that have been erroneously referred to *Bassovia*, the whole of the genus *Brachistus*, 2 species from *Chamaesaracha*, 9 from *Cyphomandra*, 10 from *Lycium*, 11 from *Parascopolia*, and no less than 250 from *Solanum*, with 134 new species, it becomes a very large genus. Yet, considering that more than a score of undescribed species have already accumulated in the herbarium of the New York Botanical Garden, and that current collections continue to add rapidly to the number, it is clear that the extent of the genus is not yet realized, and that it may vie with *Solanum* itself.

Another result that has come from Dr. Bitter's work is that of establishing the validity of small differences as possessing specific rank. With these plants, as with so many genera,

before becoming well understood, we have not known whether small differences, sometimes minute ones, under consideration, were to be regarded as specific characteristics or merely individual variations. A study of such of Dr. Bitter's recognized species as I know makes it pretty certain that, as a general rule, we must recognize such distinctions as specific, in this genus.

A few species, now classed in other genera, which apparently have not been seen by Dr. Bitter, are here transferred, and a number of new ones, from Bolivia and Colombia, are proposed.

Many others, from Guiana, Venezuela, Colombia, Ecuador and Central America, remain in our herbarium for future treatment by those concerned with the floras of those countries. The type-specimens of all species here enumerated and described are deposited in the Herbarium of the New York Botanical Garden.

*Lycianthes hispida* n. comb. (*Brachistus hispidus* Rusby, Bull. Torrey Club, 26: 198. 1899.)

*Lycianthes ferruginea* n. comb. (*Bassovia ferruginea* Rusby, Descriptions of Three Hundred New Species of South Am. Plants, 117. 1920.)

*Lycianthes Fendleri* n. comb. (*Bassovia Fendleri* Rusby, Bull. Torrey Club 26: 197. 1899.)

*Lycianthes coccinea* n. comb. (*Brachistus coccineus* Rusby, Bull. N. Y. Bot. Gard. 8: 117. 1912.)

*Lycianthes leptocaulis* n. comb. (*Brachistus leptocaulis* Rusby, Bull. Torrey Club, 26: 199. 1899.)

*Lycianthes recticarpa* n. sp. Young portions puberulent. Stems slender, much-branched, the short branchlets widely spreading, lightly angled. Leaves extremely irregular in size, on petioles 1 to 1.5 cm. long, from 2.5 by 1 cm. to 8 by 4 cm., ovate, with broadly rounded or subtruncate base and acuminate acute summit, thin, deep-green, the slender venation lightly prominent beneath, the secondaries 5 or 6 on each side, strongly ascending and lightly curved, crooked, connected by slender, crooked tertiaries. Pedicels mostly 2 together, filiform, 1 cm. long in flower. Flowering calyx-tube sub-hemispherical, 3 mm. long, 5 mm. broad, the 10 teeth equal, 2.5 mm. long, linear, thick, obtuse, erect. Corolla purple, thick, 1.5 cm. long. Four stamens half as long as the corolla (unopened), the fifth 2 mm. longer. Filaments very short. Anthers 4 mm. long, oblong, the base cordate. Style nearly as long as the corolla.



Collected by Herbert H. Smith at Quebrado del Cabo, Santa Marta, 100 feet, Aug. 26 (1876).

"A vine, to 20 ft., in swampy places below 500 feet."

**Lycianthes reflexa** n. sp. Densely yellowish-pubescent, with simple acute hairs, those of the stem, etc., divaricate or slightly retrorse. Branches elongate, slender, erect or strongly ascending, straight or more or less flexuous, terete, striate or lightly angled above. Leaves sessile, but with a short narrow petiole-like base, to 13 cm. long, and 3 cm. broad, lanceolate with long and narrowly acuminate summit, and short-acuminate base, entire, thin, yellowish-green, especially beneath, the midrib stout, prominent beneath, the secondaries, 8 or 10 on each side, very slender, strongly falcate-ascending, the remaining venation obscure. Smaller leaves mostly 1.5 to 2.5 cm. by 8 to 15 mm., oval, not acuminate. Pedicels mostly solitary, sometimes 2 together, in flower, slenderly filiform and 3 or 4 cm. long, in fruit thickening but not much elongating, strongly reflexed. Calyx pubescent with divaricate hairs, the tube turbinate-campanulate, membranaceous, with 10 green nerves, 3 mm. long, 5 mm. broad, the 10 teeth subequal, slenderly setaceous, erect, spreading or recurved, 2 mm. long in flower, becoming 3 mm. in fruit. Corolla apparently purple, 6 mm. long, divided nearly to the base. Stamens two-thirds the length of the corolla, equal. Filaments nearly as long as the anthers, which are coriaceous, brown, 3 mm. long, oval, obtuse, the pores small. Style stout, longer than the stamens.

Collected by M. Bang in Bolivia, without data (*no.* 2617 the type). Distributed and published as "*Brachistus lasiophyllus* (Dunal)" but differing from that species, as described by Bitter, in several important particulars. Also collected by the author in Yungas, 6000 ft. (Parke, Davis & Co., *no.* 2617).

**Lycianthes tomentella** n. sp. Stellate-tomentose. Stems stout but weak, terete, finely nerved or lightly angled above, sometimes reddish, the branchlets short, widely spreading, leafy at the ends. Petioles to 2 cm. long, broad but weak. Blades very unequal, from 4 by 1.5 cm. to 12 by 6 cm., oval with very slightly produced base and obtuse summit, thickish but weak, deep-green, often with reddish venation, shortly stellate-hairy and roughish, the venation coarse, prominent beneath, tomentose, the secondaries 6 or 8 on each side, widely spreading, then strongly up-curved, connected by few crooked tertiaries, the remaining venation obscure. Pedicels two to several, in flower 1 or 1.5 cm. long, thick but weak, slightly thickened upward, elongating and thickening somewhat in fruit, tomentose, like the calyx. Calyx-tube campanulate, 4 mm. long and broad, 10-ribbed, the 10 teeth irregularly unequal, to 3 mm. long in flower,

twice as long in fruit, linear, thick, obtuse. Corolla purple, pubescent, nearly 2 cm. long, divided three-fourths of the way or more. One stamen nearly twice as long as the others. Filaments very short, the smaller anthers 4 mm. long, coriaceous, lanceolate, lightly curved, obtuse, the pores whitish, on a narrow, slightly projecting summit. Style slightly exceeding the long stamen, rather stout, the stigma truncate. Only young fruit seen.

Collected by M. Bang (*no.* 630) in Yungas, Bolivia. Distributed and published as "*Solanum Sprucei* Van Heurck & Müller," and afterwards regarded as a species of *Brachistus*.

*Lycianthes pyrifolia* n. sp. (Fruiting specimen.) Branches elongated, stout, angled, flexuous, reddish-brown, more or less bristly with deciduous divaricate hairs, some, at least, of which are glandular. After the fall of the hairs, the stem is finely muricate or papillose. Petioles to 2 cm. long, stout, narrowly grooved above, like the midrib. Blades to 1.5 dm. long, 7 cm. broad, ovate, with broadly rounded or sub-truncate base and abruptly acuminate and acute summit, thin, dark-green, glabrous above, with the venation slightly prominent, the midrib sometimes minutely hairy, the principal veins of the lower surface more so, the secondaries, 6 or 8 on each side, strongly falcate-ascending, connected by few tertiaries. Pedicels 2 or 3 together, 1.5 cm. long, in fruit, slender, terete, thickened upward, pilose with whitish divaricate hairs. Fruiting calyx-tube slightly recurved, about 1 cm. broad, coriaceous, the 10 teeth 3 mm. long, linear, thick, obtuse. Mature fruit globose, glabrous, in the dried state 1.5 cm. broad, but evidently much reduced in drying, much wrinkled.

Collected by Otto Buchtien (*no.* 2816), at Espirito Santo, near Cochabamba, Bolivia, 750 meters, June 1909.

Species very near *L. hispida* Rusby.

*Lycianthes polycarpa* n. sp. (Fruiting specimens.) Finely puberulent throughout with extremely short stellate hairs. Branches thickish but weak, very flexuous, terete, reddish, finely many-nerved. Petioles 1 cm. long, grooved above, like the lower part of the midrib. Blades (only the upper seen) to nearly 1 dm. long, 5 cm. wide, ovate with rounded, very slightly produced base and abruptly short-pointed very acute summit. Pedicels mostly 3 together, the fascicles crowded toward the ends of the branches, 7 to 10 mm. long, slender, thickened upward, slightly angled. Calyx-tube rotate, about 8 to 9 mm. broad, 10-ribbed, the narrow margin and 10 teeth mostly sharply recurved in fruit, the teeth about 2 mm. long, thick, linear, obtuse. Berry globoidal, 1 cm. broad and a little longer.

Collected by the author along the Beni River, Bolivia, July 1886 (Parke, Davis & Co., *no.* 798 the type). Also at Guanai, 2000 ft., May 1886 (*no.* 784).

Species very near Buchtien's 2816.

**Lycianthes Herbert-Smithii** n. sp. Stellate-tomentellate. Stems slender, terete, finely nerved, strongly flexuous. Petioles to 15 mm. long, rather stout, flattened above. Blades of the smaller leaves 2 to 4 cm. by 1 to 2 cm.; of the larger 5 to 8 cm. by 3 to 4 cm., regularly ovate, with rounded base and abruptly short-acuminate acute summit, pale-green, very finely tomentellate above, more strongly so beneath, the slender venation prominent beneath, the secondaries 6 or 8 on each side, somewhat crooked, strongly falcate-ascending, connected by few crooked tertiaries. Peduncles solitary or two together, in flower 2 to 3 cm. long, slender, erect, the hairs longer than on the stems, those of the young fruit somewhat longer and stouter. Calyx-tube crateriform or short-cupulate, in flower 4 mm. long by 5 mm. broad, in young fruit 4 mm. long by 13 mm. broad, the teeth unequal, the alternate ones nearly a half longer than the others, 7 mm. long in flower, moderately elongating in fruit, linear, obtuse, tomentose like the tube. Corolla rotate, 2 cm. broad, bearing 5 lanceolate ribs, shortly 5-toothed. One stamen longer. Filaments shorter than the anthers, the latter lance-oblong, obtuse, 4 mm. long, the pores looking inward and a little upward. Style filiform, much exceeding the stamens, the stigma small. Young fruit tomentellate.

Collected by Herbert H. Smith (*no.* 1179), at Sierra del Libano, 6000 ft., Jan. 26, 1899, heretofore referred with doubt to *Brachistus Sanctae Caroli* Winkler, but sufficiently distinct therefrom.

"A diffuse vine-like shrub, rare in damp clearings, 4000 to 6000 ft., the flowers white."

Several additional species from Bolivia will shortly be published in my report on new species and genera of the Mulford Collection, in the Bulletin of the New York Botanical Garden.

COLLEGE OF PHARMACY,  
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## Bertoloni's Guatemalan Asteraceae

S. F. BLAKE

Antonio Bertoloni (1775-1869), for many years professor of botany at the University of Bologna, and author of one of the two principal floras of Italy, described many new species of plants from North America in the '40's and '50's of the last century. Nearly all those from the United States were based on specimens collected in Alabama by Dr. Hezekiah Gates and given to Bertoloni in 1834 by Prince Charles Bonaparte, the well-known ornithologist. Many of these have since been reduced to synonymy by Dr. Asa Gray and others. Bertoloni's much more important work<sup>1</sup> on the flora of Guatemala has had comparatively little attention from botanists. This "Florula," enumerating 79 species of which 60 were described as new, was based on a collection of plants and seeds brought from Guatemala in 1836 by Joachim Velasquez (also spelled Vellasquez by Bertoloni), who served as Mexican ambassador at the papal court. The new names here published are of course listed in the Index Kewensis and (with some omissions) in Hemsley's Botany of the Biologia Centrali-Americana, but little attempt has been made to identify them. The examination of Bertoloni's types by a botanist familiar with the flora of Central America would be sure to lead to interesting results.

Bertoloni's herbarium and library are still very carefully preserved in their original rooms at the Bertoloni home at Zola Pedrosa, some ten miles outside Bologna, by Dr. Cav. Antonio Bertoloni, the grandson of Antonio Bertoloni the elder. Dr. Bertoloni, a well-preserved old gentleman of 82, is himself a botanist, who has recently (1917) commemorated the hundredth anniversary of the calling of his grandfather to the professorship of botany at the University of Bologna by the publication of a list of unrecorded Italian lichens in the Bertoloni Herbarium. On a hot morning in July, 1925, having made the trip from Bologna by automobile, I was courteously received by Dr. Bertoloni and

<sup>1</sup> "Florula Guatimalensis," Nov. Comm. Acad. Sci. Bonon. 4: 403-443. *pl.* 36-47. 1840. The references in the Index Kewensis to this paper are to the separate ("Florul. Guatim."), with a pagination uniformly 400 less than the original. I have not seen the separate.

permitted to examine the types of all the new Guatemalan Asteraceae described in the "Florula Guatimalensis" and to obtain fragments of several for the United States National Herbarium.

The specimens in the Bertoloni Herbarium, still in perfect condition owing to the fact that they were well poisoned many years ago, lie loose with their labels in paper folders. The species of each genus are grouped in folders, and several or many genera are bound up between cardboards in packets of various sizes which stand on the shelves of the cases, and bear on the side a list of the genera contained. The exotic herbarium, the only one I had occasion to consult, is arranged by the Linnaean classes, as is doubtless the still larger herbarium, filling another room, on which the "Flora Italica" was based.

Bertoloni described ten new Asteraceae (reducible to nine) from Guatemala, all from Volcan de Agua ("Vulcano d'acqua" of Bertoloni), besides listing two others. Eight represent species which were actually undescribed at that time, but the names of two of these are preoccupied and hence unavailable. The following list of identifications is arranged in the current order of classification.

STEVIA POLYCEPHALA Bertol. Nov. Comm. Acad. Sci.

Bonon. 4: 432. 1840.

*Stevia arachnoidea* Robinson, Proc. Amer. Acad. 35: 326. 1900.

Bertoloni's *Stevia polycephala* is clearly the same as *S. arachnoidea* Robinson, described from the same locality, Volcan de Agua. Robinson's type (*J. D. Smith* 2327) has not been examined by the writer, but comparison of fragments from Bertoloni's type has been made with *Maxon & Hay* 3677 and *Pittier* 10 (of 1905), both from Volcan de Agua, as well as with *E. W. Nelson* 3649, from near the Hacienda of Chaucol, alt. 3080 m., Guatemala. Dr. Robinson informs me that he had previously recognized the identity of the two species, although apparently no published record of the fact has been made. Bertoloni's name was omitted from the Botany of the Biologia Centrali-Americana.

GNAPHALIUM SALICIFOLIUM (Bertol.) Sch. Bip. Bot. Zeit.

3: 172. 1845.

*Helichrysum salicifolium* Bertol. Nov. Comm. Acad. Sci. Bonon. 4: 433. 1840.

*Gnaphalium rhodanthum* Sch. Bip. in Seemann, Bot. Voy. Herald 310. 1856.

Bertoloni's name, apparently not used again since it was transferred without discussion by Schultz Bipontinus, is the earliest given to this species. Specimens collected by W. A. Kellerman (no. 4951) and H. Pittier (no. 46 of 1905) on Volcan de Agua, the type locality of *Helichrysum salicifolium*, are in the National Herbarium, as well as a fragment of Bertoloni's type. Both *Helichrysum salicifolium* and *Gnaphalium salicifolium* are omitted from Hemsley's Botany of the Biologia Centrali-Americana.

***Tithonia longeradiata* (Bertol.) Blake.**

*Helianthus longeradiatus* Bertol. Nov. Comm. Acad. Sci. Bonon. 4: 436. 1840.

*Tithonia scaberrima* Benth. in Oerst. Naturh. For. Kjöbenhavn Vid. Med. 1852: 91. 1852; Blake, Contr. U. S. Nat. Herb. 20: 432. 1921 (synonymy).

**SIMSIA SERICEA** (Hemsl.) Blake, Proc. Amer. Acad. 49: 393. 1913.

*Verbesina argentea* Bertol. Nov. Comm. Acad. Sci. Bonon. 4: 435. 1840. Not *V. argentea* Gaud. in Freyc. Voy. Bot. 463. 1826.

*Encelia* (? *Simsia*) *sericea* Hemsl. Biol. Centr. Amer. Bot. 2: 185. 1881.

Bertoloni's name, *Verbesina argentea*, the first that was given to this species, is not available because of its previous use by Gaudichaud for a still doubtful plant from the Marianne Islands.

**BIDENS CANESCENS** Bertol. Nov. Comm. Act. Sci. Bonon. 4: 431. 1840.

*Maxon & Hay 3690*, in the National Herbarium from Volcan de Agua, the type locality, has been identified as this species by Dr. E. E. Sherff and agrees with my notes on Bertoloni's type.

**BIDENS SQUARROSA** H.B.K. Nov. Gen. & Sp. 4: 238. 1820.

*Coreopsis trifoliata* Bertol. Nov. Comm. Acad. Sci. Bonon. 4: 436. 1840.

From Bertoloni's description and my notes and sketches of

the type, a mere scrap about 4 inches long, it is evident that *Coreopsis trifoliata* is merely one of the forms of the variable *Bidens squarrosa* H.B.K.<sup>2</sup>

SENECIO ACUTANGULUS (Bertol.). Hemsl. Biol. Centr.  
Amer. Bot. 2: 235. 1881.

*Cineraria acutangula* Bertol. Nov. Comm. Acad. Sci. Bonon. 4:  
435. 1840.

This species has apparently not been re-collected. A single head from the type and a sketch of a leaf are in the National Herbarium.

SENECIO GODMANII Hemsl. Biol. Centr. Amer. Bot. 2:  
240. 1881.

*Cacalia cuspidata* Bertol. Nov. Comm. Acad. Sci. Bonon. 4: 432.  
1840. Not *S. cuspidatus* DC. 1836.

This species is represented in the National Herbarium by several specimens collected on Volcan de Agua, the type locality of Bertoloni's plant—*Kellerman 4749, Maxon & Hay 3672, 3684, 3708*. Hemsley's type of *S. Godmanii* came from near Santa Maria, Volcan de Agua (*Salvin & Godman 327*). A single head from Bertoloni's type is in the National Herbarium. Bertoloni's name is omitted from the Biologia Centrali-Americana.

**Lycoseris crocata** (Bertol.) Blake.

*Carduus cernuus* Bertol. Nov. Comm. Acad. Sci. Bonon. 4: 431.  
1840. Not *Carduus cernuus* (L.) Steud. Nomencl. ed. 1.  
151. 1821.

*Aster crocatus* Bertol. Nov. Comm. Acad. Sci. Bonon. 4: 434.  
1840.

*Lycoseris squarrosa* Benth. Bot. Sulph. 121. 1844.

Bertoloni described the sexes of this shrub under different names, his *Carduus cernuus* being the pistillate plant, his *Aster crocatus* the staminate. Both are specifically identical with the Central American plant hitherto known as *Lycoseris squarrosa* Benth., which was originally described from "Nicoya, Gulf of Fonseca, Panama." Both of Bertoloni's names are omitted by Hemsley.

<sup>2</sup> See the discussion of this species by E. E. Sherff, Bot. Gaz. 64: 35-38. pl. 9, 10. 1917.



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Morphogenesis in Dictyostelium<sup>1</sup>

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(WITH PLATES 6-8)

## INTRODUCTION

The development of a plant from the germ cell, spore or egg, to maturity is generally characterized broadly as a process of growth and differentiation; or speaking in even more general terms, growth is made to include differentiation. Considering the matter more analytically, there is still much confusion in the technical literature and in the definitions of the textbooks as to the relative significance and the interrelations, of cell growth, multiplication or census growth, histogenetic cell differentiation, and the morphogenetic processes by which the specific form and types of symmetry of the mature metaphytic plant are produced. This is in a way natural enough, since in most plants all these processes go on more or less simultaneously and the specific significance and the functions of each are apparently inextricably interwoven with those of the others.

That all of these so-called growth processes are essentially cell phenomena has been widely recognized since the second quarter of the last century, when the cell theory was given its present-day form by the work and writings of Von Mohl, Meyen and others.

Certain simple types of slime moulds present the phenomena of cell growth, cell reproduction and morphogenesis in, so to speak, dissected form and for such cases at least afford a very direct answer to De Bary's old question as to whether the plant builds cells or cells build the plant. I have elsewhere described (1918) the processes of morphogenesis in certain coenobic algae,

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such as *Hydrodictyon*, *Gonium* and *Pediastrum*, in terms of the interrelations of free and independent cell units. The Acrasieae present a further phase of these problems in that their completed forms in the higher types of the group are erect and tree-like in their habit, with at least an approach to metameric and radial symmetry in their organization.

Olive, in his very thorough study of the group (1902) brought together convincing evidence that we have in them a very complete evolutionary series so far as form is concerned from such simple cushion-shaped masses as *Copromyxa* to the branching pine-tree-like forms of *Polysphondylium*, with its evenly tapering stem and whorls of progressively shorter branches.

I am here presenting, in microphotographs, the more essential stages in the development of *Dictyostelium mucoroides*, a form which only occasionally branches, but produces a symmetrically tapering stalk with a mass of spores at the apex, simulating the sporangiophore and sporangium of *Mucor* as its name implies. The difficulties in photographing such material are very great, and the prints I have obtained leave much to be desired. The drawings of Zopf, Brefeld, and especially of Olive, show all the essential morphological structures and the method of their development. I have, however, thought it worth while in this case, as in the case of the coenobitic algae, which I have studied from the same standpoint, to present the data in photographic form so that the measurements of size and the general symmetry relations may be shown free from any suspicion of interpretative bias. I have not attempted to free the surroundings of the plants photographed from extraneous material such as spores, minute fragments of dung which do not readily filter out of the decoction, etc. Bacteria are regularly present in these dung cultures without, however, ordinarily interfering with the apparently normal development of the *Dictyostelium*. The cultures were grown at ordinary room temperature and I am leaving also for future experimental study the effect of varying other environmental factors, such as moisture and food supply, chemical stimulation, etc.

It is to be noted also that the photographs are all, except where specifically noted, of material in the living condition. The amoebae in the creeping stages are slowly changing their form and position. It is impossible, of course, in the case of such

transparent bodies, to get photographs by instantaneous exposures. The photographs of creeping amoebae (PLATE 1, FIGS. 7-10, PLATE 2, FIGS. 17-23) were obtained by exposures of from five to fifteen seconds.

There has been wide disagreement in the use of morphological terms for the spore-bearing structures in the Acrasieae. Brefeld (1869) calls the spore-bearing organs sporanges and stalks. Zopf calls them sori and sorophores. Thaxter calls the similarly formed structures in the Myxobacteriaceae cysts and cystophores. The term cyst has been used very loosely. There is no question that it should be retained for those more or less transitory dormant stages of interrupted life activity which may intervene at any period in the life cycle and in which the organism encloses itself in a thick sheath.

Brefeld reports that encystment of the myxamoebae may occur at any stage in the development of the culture. He describes the process and the germination of the cysts quite fully but was unable to determine the conditions which bring it about. Olive states that encystment is brought about by slow drying. Zopf says that microcysts are formed under unfavorable nutritive conditions.

The use of the term cyst in the literature of the protozoa is even more confused by the fact that the term has been used for stages in which sexual reproductive processes are involved. This literature has been recently summarized by Kater and Burroughs (1926). It seems clear, however, that encystment is not in *Dictyostelium* a recurrent phenomenon in the life cycle in the same sense that the spore-bearing structures are, and if the term cyst is to be retained for the non-cyclic dormant stage, we should not use the same term for the spore-bearing structures. The term sorus, used by Zopf, is also variously and loosely used in mycological terminology. I shall use it for the spore mass to avoid the danger of confusion involved in the use of the term cyst. The stalk is then the sorophore, and I shall call the entire spore-bearing structure the sorocarp as such a term is much-needed in the description of the morphogenetic processes with which we are concerned. The amoebae for clearness should certainly be distinguished as myxamoebae, though convenience may lead to occasional abbreviation of the term. There are certainly adequate grounds for distinguishing these fruiting

structures, which are formed primarily by the morphogenetic aggregation of free cell units, from spore-bearing structures such as those of the mycelial fungi which are formed by the combined and simultaneous processes of growth and cell, or at least nuclear, multiplication. The latter are regularly regarded as growth forms. These more special types may be distinguished as aggregation forms, or by an extension of its current significance, morphallactic forms. With them should be included also those coenobic algae whose colonies are formed by aggregation of free swarming zoöspores. The view that the Acrasieae are primitive slime moulds seems to me to be supported by abundant evidence, and while in the latter the development of a true plasmodium, with its extended period of growth and nuclear division, tends to break down the limits of the category, in my opinion they also may be classed as aggregation forms.

#### STRUCTURE OF THE SOROCARP

*Dictyostelium*, as noted, has an easily recognized resemblance to a *Mucor*. I shall describe first the general facts as to the range in form, size and proportions of the *Dictyostelia* and then take up the data as to their life history and form development. The general form and proportions of an average specimen as grown in a hanging drop of dung decoction is shown in FIGURE 1. The outlines of the figure are by no means sharp. It is practically impossible to photograph these minute plants either on their natural substrata or as they grow erect in moist chambers. The working distance of the lenses is far too short.

It was early found, however, that when grown in hanging drops, the myxamoebae build the stipe downward only a short distance and then curve out horizontally. This is not in agreement with Brefeld's statement that the sorocarps grow out at right angles to the substratum, no matter how the latter is oriented. With the use of the very low magnifications given by the microplanars, it is possible to get figures appearing more or less as if the whole sorocarp were in one plane. The rather bulbous base is rather over emphasized in density, but the taper of the sorophore and the ratio of the diameter of the spore mass to the height of the whole plant are not seriously misrepresented.

The sorocarps shown in FIGURES 1 to 6 were photographed in this way. They were chosen to represent the wide range in

size and proportions which *Dictyostelium* shows. Its range of variability in these respects is certainly of the same general order as is found in other fungi and in the higher plants. The ratio of height to diameter of sorus in the plant shown in FIGURE 1 is about 10 to 1; the corresponding ratio in the plant shown in FIGURE 3 is about 2.3 to 1. In the two sorocarps in FIGURE 2 it is about 8 to 1.

These variations in size and proportions are doubtless due largely to environmental conditions, as in the higher plants, but as will be noted, there are certain problems in connection with what we call normal size in a species which can be studied experimentally to especial advantage in these forms.

In FIGURE 2, two small plants are shown which were growing rather close together in the culture and whose sorophores and sori lie nearly enough in the same plane so that they both appear in the photograph. The two sorocarps not only curve in a vertical plane by negative geotropism, but also in the horizontal plane, both bending in about the same direction to the left. The proportions of height to diameter of sorus in these two plants are about the same (8 to 1) though the sorocarps are quite unequal in size.

All five of the sorocarps (FIGS. 1 to 5) were growing scattered in hanging drop cultures, the bases of the sorophores attached to the microscope slide and immersed in the dung decoction. It is very common in hanging drop cultures to find the sorocarps at the margin of the drop as is shown in the case of those sorocarps photographed in successive stages of development and shown in FIGURES 18-21. In what are perhaps the most typical sorocarps, the sorus is globular, no matter from what angle it is viewed. Its form would thus seem to be the expression of simple surface tension relations. It is to be remembered, however, that at no time in its development, is it a continuous mass of protoplasm, much less a homogeneous liquid. Throughout its development it contains the upper portion of the sorophore, which extends as a relatively solid axis from its basal to its apical pole. In its earlier stages it is made up of the closely packed plastic bodies of the creeping myxamoebae, each maintaining, however, its own individual plasma membrane. The myxamoebae, in the process of ripening, transform themselves into fairly thick-walled spores which are oblong bodies about

twice as long as wide. This transformation of the amoebae into resting spores takes place so far as known without any cell division occurring. I have not yet completed the cytological study of *Dictyostelium*, but have so far seen no clear evidence that the amoebae divide at any time during the production of the sorophore or sorus.

The amoebae are surrounded individually and as a mass with more or less slime, and this forms a continuous thin film around the whole sorus when mature. In my observation, however, it never forms a membranous layer comparable to a sporangium wall. It is not so abundant that the whole mass could be regarded as a colloidal aggregate, the slime forming the continuum of a two-phase system.

I am inclined to the opinion that the globular form of the sorus is the expression of an active tendency of the amoebae to crowd together as closely as possible. I have emphasized that this globular form seems to be most typical for the species. It is, however, quite common to find that when viewed from the side the sorus is slightly lemon-shaped, with a conical papilla at its vertical pole. This papilla is occupied by the apical end of the sorophore and seems to be the expression of a tendency of the sorus to drag downwards by its own weight on the sorophore which supports it. The base of the sorus in FIGURE 1 also seems to extend downward a trifle around the sorophore. The sorus alone, when regard is not had to showing the sorophore, can be photographed in these hanging drop cultures with the greatest ease and at considerably higher magnifications. FIGURE 4 shows such a view of a sorus suspended in air on the sorophore which is attached far enough below the median plane of the sorus so as not to appear in the figure. The outline of the sorus is perfectly circular, and in view of its highly inhomogeneous makeup is a good example of biological economy of space however its form is achieved.

The base of the sorophore is regularly, as the photographs show, somewhat bulbously enlarged. The sorophore tapers much more rapidly at first than higher up, quite as is the case with the stems of higher plants. The method of support and anchorage in *Dictyostelium* is quite specialized and peculiar to the group. The stipe does not radicate downward. It is embedded in a very considerable mass of slime which adheres to the



substratum and tapers upward enclosing as a sheath the enlarged base of the sorophore. However, owing to the density of the mass, I have not been able to get adequate photographs to show the relations of its parts.

The figures as given illustrate a few of the variations in size and form of the sorocarps as I find them. It is clear that in having an erect, tapering and sometimes branching axis terminating above in a fructification and below in a base specialized for anchorage, *Dictyostelium* has in its form the type of organization which is common to most plants from the fungi and filamentous algae to the higher plants. It lacks only a branching anchorage system to simulate a true cormophyte in its form.

In *Polysphondylium*, with its whorls of rather regularly spaced branches which are progressively shorter toward the apex, the differentiation of nodes and internodes is at least simulated. It is interesting in the light of the methods by which this structure is developed that it suggests so obviously what Child (1915) calls differentiation by axial metabolic gradients, though there is no possibility that the cells at different heights in the sorocarp represent successively different growth stages.

In these stalked forms we have also a typical differentiation into soma and germ plasm, the sorophore being wholly somatic and the sorus wholly reproductive. It is further to be remembered, as noted above, that the Acrasieae include an evolutionary series, beginning with forms in which there is no differentiation of soma and germ plasm, as *Copromyxa*, in which all the cells ultimately become reproductive spores, and culminating in the stalked forms like *Dictyostelium* and *Polysphondylium*, in which the differentiation of soma, the sorophore and germ plasm, the sorus is quite as definite as in the most highly specialized organisms. *Dictyostelium* represents thus a distinct advance in its life cycle and degree of differentiation, beyond such coenobitic algae as *Hydrodictyon*, *Gonium*, and *Pediastrum* in which every cell, barring accidents, ultimately becomes reproductive.

#### CELL GROWTH AND MULTIPLICATION

The stages in the life history of *Dictyostelium* have been fully worked out by Brefeld (1869 and 1884), Van Tieghem (1880) and Olive (1902).

If spores are sown in a drop of dilute dung decoction some of

them may germinate at room temperature within a day or two, and germination may continue for several days. The spore wall is thrown off and the protoplast creeps away as an amoeba. These amoebae divide apparently by constriction. I have not studied the process from the standpoint of its method nor the behavior of the nuclei. Brefeld describes the germination of the spores, and figures very fully the appearance of the amoebae and their division. I have photographed the amoebae at various stages. In FIGURE 7 several of them are shown as they appear when not creeping very actively in any specific direction. Very numerous bacteria are present and appear singly and in clumps among the amoebae. The latter are irregular in outline, indicating pseudopodial activity, but were decidedly sluggish in this particular preparation.

FIGURES 8, 9 and 10 show the myxamoebae in a much more active condition. They are much elongated owing to the pseudopodia being thrust out chiefly in one direction. This is the habitual form of actively creeping myxamoebae, and indicates that they tend to travel in a certain direction for a longer or shorter period, rather than to move aimlessly in this and that direction as shown in Brefeld's figures. The end of the amoeba on which the pseudopods are being thrust out can generally be recognized by its vagueness of outline and appearance of being out of focus. This is of course due to movement during the exposure of the photographic plate. The time of exposure of these figures was from 5 to 15 seconds. This elongated form of the amoebae is characteristic when they come to the aggregation stage and are all creeping more or less directly toward the point at which the sorocarp is being built. At this stage, however, a study of these figures (FIGS. 7-10) shows that, while at certain points the amoebae are lined up as if following each other in a definite direction, in general their long axes show every possible orientation. They are creeping about, feeding and dividing, giving no evidence of any tendency to act in common. I have called attention to the elongated forms of these amoebae but a glance at the figures shows that it is by no means universal, and that many of them show what is commonly described as irregular amoeboid form. Certain of the elongated forms are bent at greater or less angles. The common method of changing direction is not apparently by thrusting out a pseudopod from

the middle, but by changing the direction of the pseudopods at what for the time is the anterior end of the amoeba. I have not determined as yet the number of cell generations through which this independent creeping, feeding and dividing stage persists. Many interesting problems present themselves in this connection as to the relative cyclic behavior of a culture started with one or few spores as compared with those started with many spores, and as to the effect of scant and abundant nutrition and of different kinds of foodstuffs and other chemicals on the length of this vegetative reproductive cycle. As noted above, the experimental aspects of these problems of growth and morphogenesis are left for a further contribution. With the completion of this feeding, growing and dividing stage we have a swarm of independent cells which next proceed to build the sorocarp.

The ontogenetic processes of cell growth and multiplication are thus sharply separated from those of morphogenesis, and there is no possibility of confusion as to the general interrelations and functions of both types of activity in the production of a symmetrically organized multicellular plant body. Growth and cell division are first completed as independent processes. Cell differentiation and morphogenesis follow as a further series of independent processes.

#### EARLY AGGREGATION STAGES

As noted above, the sorocarps may be formed at the margin of a hanging drop or scattered irregularly through it. There seems, however, to be a marked tendency in these forms as in the *Myxomycetes* to seek out the relatively dry regions of the substratum when they come to the fruiting stage. Sorocarps formed on the margin are especially favorably placed for study and for photographing, since the culture substratum, whatever it may be, is especially thin and transparent in that region. The determining factors in the selection of the point at which a sorocarp is to be formed are not easy to make out. The sorocarps are by no means evenly distributed around the margin of the drop. There is no evidence that one-sided illumination or slight variations in temperature affect the matter. Conditions too subtle for our ready discrimination may be determinative.

When, however, a clump of the myxamoebae are found to-

gether, it is at once obvious that, in a considerable radius about them, the remaining myxamoebae orient their movements so as to creep toward the group. Here again the stimuli which determine this specifically oriented movement are not at once obvious. It is not clear whether those which start later are influenced by the existing group or move toward that region because of the same stimuli which guided those which became the first members of the group. In FIGURE 17 an early stage in this grouping is shown and it is at once obvious that the movements of the amoebae are, at least at first, not oriented with any great exactness upon a specific point. The outline of the margin of the culture drop is not at all clear in this figure. It was not an even arc of a circle as in FIGURES 18-21, but was very irregular as in FIGURES 22 and 23. Subsequent observation showed that in this case the sorocarp was built in a projection of the culture drop a little to the left of the center near the top of the figure. A considerable number of amoebae in this region form an oblong group parallel to the outline of the culture drop. The group thins out to the left. To the right its outlines do not come out in the reproduction. Radially inward from this group are two very irregular clumps of myxamoebae. The general orientation of most of them is toward the point where the sorocarp will be formed, but the orientation is not at all mechanically exact.

FIGURES 18 to 21 are from photographs of another sorocarp at successive stages in its formation. They were taken at intervals of fifteen to twenty minutes and show the process of aggregation from a very early stage up to the time when the sorophore is well started and the young sorocarp has pushed up out of the culture medium so that the light from the side strikes it and is reflected upward giving the high light spot near its vertex.

In all these figures as noted the margin of the culture drop is sharply outlined as an arc of a circle cutting across the figure above its middle and the habit of the groups of amoebae to push out onto the glass slide carrying the margin of the culture drop with them is well shown. The effect is to give the group an even drier position in which to start the sorocarp, and is probably an expression of negative hygrotropism at this stage in their development. The sharply rounded outline of the projection is doubtless a matter of surface tension in the liquid culture medium, together with the tendency of the amoebae to crowd together

into as small a space as possible. Such conditions as this show a strong contrast to those shown in FIGURES 7-10 where the amoebae in general are scattered irregularly through the culture drop while feeding, growing and multiplying by division. The change shown is doubtless the expression of cyclic changes in the myxamoebae themselves and may be classed with what are generally regarded as phenomena of maturity. No sexual reproduction has yet been discovered in the group, but the periodic recurrence of this tendency to aggregate is perhaps comparable in a way to the periodic recurrence of the condition which leads to sexual reproduction in forms in which the latter process occurs.

FIGURES 18 to 21 are somewhat less magnified than FIGURE 17, but the elongated form of the amoebae and the orientation of their long axes in the direction toward the forming sorocarp is evident. They are obviously moving both in groups and singly toward a common point where they pack themselves closely together into a rounded cushion-like heap.

As noted, the nature of the stimuli by which the amoebae are guided in forming these aggregations is not obvious. They show some tendency to form radial series as if they were each following a path marked by their predecessors. This arrangement is, however, by no means universal. Many amoebae are scattered as isolated individuals and none-the-less obviously from the orientation of their long axes are seen to be moving quite directly toward the forming colony. This orientation is, however, by no means mechanically perfect. Some individuals are turned a little to the one side, others to the other; an occasional individual is placed quite transversely to the direction toward the group. The positions of the cells show free individual variations but with a very marked tendency to oriented movement in a direction toward the site of the future sorocarp.

IN FIGURE 18, as in FIGURE 17, there are quite irregular clumps of amoebae just back of the centre of aggregation. Outside the region immediately adjacent to the group this figure does not show the amoebae or shows them very faintly. This is, however, a matter of the inadequacy of the photograph. The whole region included by the figure and even further out showed numerous scattered amoebae with more or less definite orientation of their long axes toward the young sorocarp.

IN FIGURE 19, oriented amoebae are shown at much greater

distances from their goal, but here again the photograph leaves much to be desired. The most active amoebae are naturally more blurred than those which are less active. The margin of the group shows a radiating outline, indicating that the myxamoebae maintain their elongated form as they join the mass.

In FIGURE 20 a condition has developed which is of frequent occurrence in connection with the formation of sorocarps on the margin of the culture drop. The amoebae coming in from the left of the group, along the margin of the culture drop, are so numerous that they form a plasmodium-like strand or stream in which they advance by a sort of mass creeping movement toward the young sorocarp. This condition regularly arises in very abundant and well nourished cultures. All of Brefeld's figures show this condition, especially in the region near the forming sorocarp. Further out at the ends of these massed radiating strands he shows the amoebae creeping together more individually to form the strands.

In my cultures these strands are produced as a result of the tendency of the maturing amoebae to creep toward the margin of the drop. This tendency represents an early phase in the fruiting process. They first creep toward the margin of the drop and then converge on the points where the groups are forming. The result is that there are proportionally many more amoebae coming in from the marginal region than from the region radially inward from the group, and these marginal amoebae naturally become aggregated into more or less massive strands. Even if they are not so numerous as to make these confluent masses and still continue to creep individually toward their goal, they are none-the-less likely to be more numerous along the margins. In FIGURE 22, which shows this condition, there is a fine illustration of their interrelations and the general method of their advance to the sorocarp. There is in this case a distinct swarm of them strung out at a slight angle to the, in this case, rather irregular margin of the drop, all headed more or less directly for the group but all behaving quite as independent individuals. There is no more tendency in this case to form a pseudoplasmodium than there is in a swarm of minnows. They show no evidence of following each other directly, though of course it would be hard to prove that each individual might not be following a trail left by some earlier predecessor.

FIGURE 21 represents the last stage photographed in this individual series. The increased blackness of the central group indicates its greater height and opacity. The pronounced high light spot on its vertex indicates that it was well above the surface of the medium so as to reflect the light coming in from the side. There are not many amoebae about it and those that are shown are not so pronouncedly elongated as in earlier stages. The group had doubtless suffered somewhat from the disturbances involved in photographing.

FIGURES 22 and 23 represent stages from the development of other sorocarps selected to show particular conditions. I have referred above to the evidence of individual independence in the movements of the amoebae as shown in FIGURE 22. In FIGURE 23, the development of the sorocarp was checked for some reason at a stage about the same as that shown in FIGURE 21. The difference in appearance of the amoebae in the two figures is obvious. In FIGURE 23, they are angular or rounded and about isodiametric instead of elongated as they are in all the figures in which they are shown in the active creeping condition. In coming to rest the true nature of the pseudoplasmodium becomes obvious. In rounding up, the amoebae draw away from each other, and the fact that they have not fused, as they do in a true plasmodium, becomes conspicuous.

The series as a whole (FIGS. 17-23) illustrates the essential facts as to the method by which the amoebae congregate as free individual cells, each responding for itself and independently to the stimuli which direct this highly specific morphogenetic process. I have referred to the fact that, in Brefeld's and Olive's figures, there is the possibility of interpreting the streaming together of the amoebae as a sort of massed or plasmodial motion, more directly analogous to that in the Myxomycetes. I have studied especially these less crowded cultures, for the sake of making it clear to what degree the coming together of the amoebae in pseudoplasmodia is necessary for, or directly associated with their oriented movement. It seems to me that the distribution and orientation of the amoebae in all the figures (17 to 23) make it clear that the individual cells, as such, are the active and self-determining units in the whole process of coming together to form a sorocarp. In the cultures, the morphogenetic process in these early stages is initiated and carried out by the

cells as such, without any confusing element of mass or aggregate action which might afford opportunity for assuming the existence of some organizing principle, the property of the mass as a whole in accordance with which the morphogenetic process was being carried on. There is no question that the phenomena of true plasmodial movements, and the morphogenetic processes as seen in the Myxomycetes, are regarded as strong evidence in favor of those conceptions of growth and morphogenesis as mass phenomena of protoplasm, rather than activities of individualized cells which Sachs has emphasized.

The formation of the sorocarp in the Acrasieae, by strictly individualized action of the cells, shows most interestingly that there is no occasion for assuming any new method of morphogenetic control in the case of the Myxomycetes beyond what is in evidence in these pseudoplasmodial forms. I have, however, studied crowded cultures like those of Brefeld and Olive, and have prepared even more extreme cases. If the spores are sown abundantly, the amoebae may become so numerous as to form a continuous mass over the glass. FIGURE 16 is a photograph of a small region in such a culture. The amoebae form a nodular and even ridged thick film in which the individuals glide over and around each other but show no connected streaming movement such as is so characteristic of a true plasmodium. The character of the movement affords evidence of the independence of the individual amoebae as Zopf (1885) has illustrated it in his very crude diagram of a pseudoplasmodium. The lower righthand corner of the figure extends into the marginal region of the culture where the depth of the culture medium is less and toward which, as noted above, there is a specially directed movement of the amoebae in the cultures approaching maturity. The effect is in this densely crowded culture to form a thick corrugated or ridged zone which extends around the whole periphery of the culture. The figure shows an arc of this zone extending diagonally across its lower righthand corner. The roughened surface and marginal ridged zone could only be brought out adequately by the use of reflected as well as transmitted light, and hence the magnification used had to be very low.

This culture had reached the fruiting stage and the figure (FIG. 16) shows two young groups of myxamoebae, rather close together. They lie on a diagonal line a little above its center



and appear as two prominences connected by the faint indication of a ridge of amoebae which is more conspicuous near the smaller left-hand group. The creeping movements of the amoebae around and between these two young sorocarps were very active and illustrated a situation not uncommon in crowded cultures but relatively rare in those which are not so densely populated with myxamoebae. The ridge between the two young groups indicates a general tendency of the amoebae to move toward the region of the two groups indiscriminately, without directing their movements specifically toward one or the other. In the immediate region of the ridge the movements were indicative of a conflict of stimuli with a fluctuating variable result. This particular culture was observed for fifteen to twenty minutes, during which it was alternately placed in the damp chamber and on the stage of the microscope for observation, when it became so dry that movement ceased without either *anlage* developing into a mature sorocarp. Movement in the ridge itself was at times toward one and again toward the other of the groups, though such movements never extended entirely to the group away from which the movement was proceeding. The movement on the two sides of the ridge was at times in the same and at other times in opposite directions. The whole picture indicated uncertainty as to whether both or only one of the groups would become a complete sorocarp and was a convincing proof of the delicacy of the stimuli to which the amoebae respond. It is not uncommon to find two completed sorocarps as close together as the two young groups in this figure, but in such cases as a rule one sorocarp is much smaller than the other and was evidently formed later by amoebae which came together after the larger sorocarp was completed or well advanced in its development. The two extremes of monospore cultures on the one hand, and these sown with great masses of spores on the other, need careful comparative study under controlled conditions and with variation of environmental conditions.

#### FORMATION OF THE SOROPHORE AND SORUS

The stages immediately following that shown in FIGURE 21 are particularly difficult to obtain under conditions making it possible to photograph them. The mature forms, as shown above, can be photographed in hanging drop cultures with low mag-

nification, but I have found no way of photographing the intermediate stages *in situ* with sufficiently high magnification to bring out the desired details. I have had to resort to the method of pushing such stages over and covering them as they lie in the culture medium with a cover glass. This of course results in great disturbance of the masses of myxamoebae in their relation to the sorophore and the slime masses which they secrete and in which they are more or less imbedded. However, the observations of Brefeld (1884), Olive (1902), Zopf (1885) and Van Tieghem (1880) agree as to the essential facts for these stages and the photographs I have obtained confirm the figures and descriptions of these authors. Olive's figures are especially clear and grammatical.

The myxamoebae which form the initial group, compact themselves into a rounded mass, the sorophore initial, imbedded in slime. The anchorage of the mass and the maintenance of its orientation to the substratum are effected by the slime which the amoebae have secreted. When once this base of the future sorocarp is established the amoebae proceed to pile themselves upon its vertex and build it higher and higher. The primary orienting stimulus in erect cultures, it seems to me, is that of maximal resistance to motion, without, of course, loss of contact with their fellows. The amoebae glide in that direction in which the greatest resistance is offered to their motion. We may call this the stimulus of maximal resistance and its obvious relation to the principle of functional hypertrophy is at once suggested. As shown in inverted cultures, negative hygrotopism is also involved. This reaction brings them, even in the earliest stages, to the exact apex of the rounded mass produced by their primary movements of aggregation. Those myxamoebae which succeed in reaching a certain area of maximum downward pull have reached apparently a position of equilibrium and proceed very gradually at first to build rigid walls and pass into a condition something like encystment.

Up the stalk so formed the mass of amoebae, as they arrive, continues to climb, and at its vertex they contribute themselves to its further elongation upward. The amoebae at these stages are regularly numerous enough to form a pseudoplasmodium and the entire young sorophore is enclosed within it. As it becomes still higher its apex remains enclosed in an oblong pseudoplas-

modial mass. Below this, however, the pseudoplasmodium narrows to a rounded strand applied to one side of, or spirally coiled around, the sorophore but not enveloping it on all sides. I have obtained a figure of this stage (FIG. 11) which shows a segment of the sorophore made of the compacted amoeboid cells to the right and the pseudoplasmodial strand attached to it on the left. The pseudoplasmodial mass shows faintly the outline of the individual amoebae, though as the preparation was much disturbed and some minutes intervened in its preparation for photographing, it would not be safe to assume that these appearances are normal or represent the shape of the myxamoebae as it is in the actively creeping mass.

A comparison of the cells making up the sorophore at this immature stage with those in the ripened sorophores shown in the figures in PLATE 8, shows that at this stage they are more rounded, less flattened upon each other, and give less the appearance of a typical parenchyma. It is evident that the achievement of their definitive forms by the cells of the sorophore is a slow process. In this figure the thin sheath of dense slime which envelopes the sorophore can be recognized as a continuous structure, especially at the points where it bridges the gaps between those cells which are so rounded in outline as not to fit closely together.

This zone of uncompacted cells which still retain their capacity for amoeboid changes in form is apparently relatively longer in the later stages of sorophore formation. The fact that in the figures (FIGS. 11 and 12) the cells are rounded rather than amoeboid in outline, together with the bends and irregularities in outline of the sorophore in the same figures, is quite possibly due to disturbance during the preparation for photographing. FIGURE 12 shows the same young sorocarp in a region a little higher up where the pseudoplasmodial strand is just widening out into the oblong apical mass. The sorophore occupies the median vertical axis of this mass and at once supports it and the pseudoplasmodial strand as it continues to glide upward. This figure is much less magnified than FIGURE 11.

FIGURE 13 shows a short segment of this apical pseudoplasmodial mass with the young sorophore in its centre. The magnification in this case is approximately the same as in FIGURE 11. In this figure the cells are still more rounded and less compacted than in FIGURE 11, and the thin slime sheath of the soro-

phore is well shown. It is obvious that as the sorophore reaches greater height the whole structure becomes more and more unsteady, and the possibility of maintaining a position of maximum resistance to downward pull becomes more and more difficult. The myxamoebae, by bending of the whole sorocarp or by shifting of the pseudoplasmodial mass, are brought into new relations to the downward pull and prevented from keeping the equilibrium point. They are thus compelled to carry on for longer and longer periods those delicate readjustments of form and position which give the gentle upward curves of the sorophore and maintain the balance of the pseudoplasmodial mass. These readjustments may be characterized as functional morphallaxies and constitute the most delicate of the morphogenetic responses by which the symmetrical and adaptive form of the sorocarp is achieved.

FIGURE 14 shows the apex of a young sorocarp at about this stage of development. The more or less rounded outlines of the individual amoebae are quite definitely shown over the apical surface and further back, though here again it would not be safe to assume that the living cells have not changed their form as the result of the manipulation. This figure shows clearly the trumpet-shaped widening of the sorophore sheath at its upper extremity. This form of the sheath has been observed by Brefeld (1884), and Olive (1902). In such stages it would seem that the sheath as first secreted by the amoebae may be much wider than its later diameter and that it narrows gradually as the cells within take on more and more their permanent form and space relations in the ripe sorophore. A cytological study of stained sections of this as well as other structures and stages in the formation of the sorocarp may be expected to bring out further structural details which may make more clear both the nature and the origin of the sheath.

We are particularly in need of light on the nature of the creeping process by which the pseudoplasmodium raises itself upon the stalk which it simultaneously is building at its upper end. The behavior as to form changes, etc., of the myxamoebae in a pseudoplasmodium, as compared with the pseudopodial motions they show when creeping as free individuals, needs much further study. That we are still far from agreement as to the cell structures and their relations and functions in ordinary pseu-

dopod formation in the larger amoebae is clear from Mast's (1923) recent new analysis of the whole process and his very interesting conclusions as to the relations of the various surface layers of the cell to each other and to the formation of pseudopods in the process of creeping.

As the height becomes still greater the possibility of maintaining a position in the centre of downward pull reaches a limit and the myxamoebae give up the effort and "consent to be elected to the germ plasm." This critical point, when the capacities of the myxamoebae for functional morphallaxis are overtaxed may, of course, be reached at various points according to the total size of the pseudoplasmodial mass: when the upper cells of the sorophore are still approximately isodiametric; when they are horizontally flattened discs; or when they have become vertically elongated in a ratio sometimes as high as 1 to 4 or more. In a given environment this will be a matter of the relation of the diameter of the sorus to the cross-section of the sorophore. We find thus a natural self-limiting factor, an organic regulation without entelechie, which determines the height of the sorocarp. An adaptive result is thus achieved in an entirely indirect and incidental fashion. The capacity to respond to the stimulus of maximal resistance has no direct and necessarily natural connection with spore dispersal as such.

The final stages in the formation of the sorocarp consist in the cessation of sorophore formation under the conditions just described and the completion of the ascent of the pseudoplasmodial mass upon it. The rounded pseudoplasmodial strand shown in FIGURE 11 withdraws upward until it forms a part of the oblong apical mass. This mass gradually contracts upward until it forms a more or less perfect globe on top of the sorophore as shown in FIGURES 2 to 5, or the slightly lemon-formed type with apical papilla shown in FIGURE 1. These variations are due perhaps to differences in the degree of favorableness for the completion of the ripening processes.

The number of spores contained in a sorus can be determined rather readily by bending over a sorocarp just before maturity till the sorus touches the glass substratum. The spores then spread out in a film with the sorophore in the midst and can be readily counted. FIGURE 15 shows a photograph of such a preparation. The spores extend out in a projection at the upper

left following an extension of the film of moisture in which they lie. The number of spores in this case is approximately 1,300. The possibility of readily determining in this way the spore number produced in any given sorus and culture is of significance in connection with the problems of life cycle as affected by the initial spore number sown which were referred to above.

We can distinguish roughly three regions of the sorophore which differ in the rate at which it tapers. This is more or less obvious in FIGURE 1, though the outlines of the sorophore are too vague to permit accurate measurements in this case. First, there is a short basal region in which the diameter diminishes very rapidly. This is the more or less bulbous base. Second, there is a median region in which the reduction of diameter goes on much more slowly and in which there may be regions which are almost perfectly cylindrical. This portion corresponds to that of a tree trunk above the radiating base and extending in forest grown trees to the branches or further. Third, we have the terminal region in which the sorophore tapers more rapidly and in which we may even find its individual cells vertically elongated. I have determined the ratio of length to width in a series of photographs of parts of sorophores and in PLATE 8 have reproduced a number of figures illustrating the degree of tapering in form at various heights and from sorocarps of various sizes.

FIGURE 24 shows a segment from near the base of a rather large sorophore and is a massive parenchymatous aggregate. It is impossible to get more than a surface view of the outlines of the cells in a photograph, since the tissue is too dense to show anything in a median optical plane. However, enough is shown to indicate that the cells in general take the theoretically to be expected surface tension form and show in optical section from five to seven or eight sides. In the center they might be expected from the method of their aggregation and getting their shape by mutual pressure to appear as the tetrakaidekahedrons which least surface conditions would require (Lewis, 1923).

In a region where the sorophore has from four to six cells in cross-section (FIG. 25) as would be expected the cells are still approximately isodiametric. The taper in this figure is approximately at the rate of 1 to 112; that is, there is a reduction of 1 unit in diameter to every 112 units in length. This is a slow rate of decrease, and that it is maintained with comparative constancy

through considerable series of cells shows how delicate the responses must be by which it is achieved. There can be no recourse in such a case as this to the assumption that this gradual tapering is the expression of surface tension in the whole sorophore. It is achieved, just as it is probable the tapering form of plant stems and trunks generally is achieved, by the specific response of the living cells to factors of their external and internal environment. It is at once obvious that there can be no principle of organization operating in the whole swarm of myxamoebae to determine the form of the sorophore as a whole.

The sorocarp is not formed as an evolutionary unfolding of a growing mass but by the spontaneous assembling of free independent and mature cell units, whose capacity for delicately adjusted response to stimuli gives the mature structure its symmetrical and highly adaptive characters. As noted, morphogenesis is here shown as an independent process in no way dependent on growth or cell division, further than that these processes furnished the cells which carry out the morphogenetic processes.

The number of cells in the cross-section of the sorophore diminishes gradually with its tapering form. In FIGURE 26 we have a segment in which the number is reduced to two or three in its basal end and diminishes still further to two and then one at its upper end. The cells at this level in the sorophore tend to become wedge-shaped and flattened horizontally instead of remaining isodiametric as in the thicker portions of the stipe further down. The result in median optical section of the sorophore is to give somewhat the appearance of saw teeth interlocking with each other. The cell walls form a rather conspicuous more or less regular zig-zag line down the middle of the sorophore.

At the upper end of the segment shown in FIGURE 26 there is a rather sudden narrowing of the sorophore in diameter and a corresponding reduction in the number of cells in cross-section from two or three to one. The taper from the basal region to the region just below this more sudden narrowing is at the rate of about 1 to 103. If we include the whole length of the segment the rate of narrowing is about 1 to 48. The transition from a cross-section of two or three cells to one is perhaps a critical point and may involve more rapid change in the rate of tapering.

Not all sorophores show such an even rate of tapering as is indicated by such cases as those shown in FIGURES 25 and 26. In FIGURE 27 we have a segment of about the same general character as that shown in FIGURES 25 and 26, but it shows no such regular tapering form. Its fluctuations in diameter as visible at once to the eye are not great, but they are of an order of magnitude comparable to those which constitute the normal tapering itself. Basing the estimate on measurement of the diameters at the two ends of the segment, the taper would be 1 to 533, but in such a case these measurements can have little significance. Another segment of about the same character as to the number of cells in its cross-section and which is not so irregular as the one first considered, is shown in FIGURE 28. This segment of sorophore shows almost no measurable taper and illustrates the tendency in the median region of the sorophore to show a very slight or no progressive reduction in diameter for considerable distances.

FIGURES 25 to 28 are all from different sorocarps, and illustrate the variation in tapering and shape of the cells which characterize the middle region of the sorophore. The remainder of the figures belong to the upper or terminal region. We can consider them best in two groups. FIGURES 29, 30 and 31 are characteristic of wild growing large sized sorocarps. FIGURES 32 to 36 are from the less well nourished and much smaller sorocarps grown in hanging drop cultures such as were used for the study of the behavior of the individual amoebae in the growth and aggregation stages as described earlier. The two groups are quite different in the shape of their cells. In the second group the cells are much elongated vertically, while in the first they show no such elongation and are regularly disk-shaped, their transverse or horizontal axes being longer than their vertical axes.

In FIGURE 29 we have a segment of the terminal portion of a sorophore in which the number of cells in cross-section fluctuates from one to three. Those cells which fill the entire cross-section have transverse diameters two to three or more times their vertical diameters. In the cases where two cells fill the cross-section they tend to be wedge-shaped. The taper in this section is 1 to 67, much more rapid than that in the middle region of the sorophores as shown in FIGURES 25 to 28.

In FIGURE 30 is shown a portion of a sorophore made up of



disk-shaped cells of almost uniform size, with their width, the diameter of the sorophore, uniformly greater than their height. Near the middle of the segment a cell is broken and the transverse walls of the adjacent cells are curved out toward it, showing that turgor is still present in these cells even in the mature sorophore. This sorophore segment shows no easily measurable taper, a condition much less common in the apical than in the median region.

The part of the sorophore included in the sorus is sometimes less even in outline than that below. This condition is illustrated in the terminal segment shown in FIGURE 31. In this case the apex of the sorophore is turned to one side. Back of this it swells slightly and then narrows again. Lower down a cell is broken or missing as in FIGURE 30 and there is the corresponding outward curvature of the walls of the adjacent cells. The segment is made up of a single series of cells as in FIGURE 30, but they are less uniform in shape and size. The transverse septa are quite diagonally placed in a number of cases. There is nothing on the sorophore to mark the point at which it passes out of the sorus in these figures. The slime which coats more or less all the parts of the sorocarp has no definite significance as a peridium such as we find about the spore mass in a myxomycete.

The remainder of the FIGURES 32-36 are as noted from much more slender and smaller sorocarps. In FIGURE 32 we have a segment made up of disk-shaped cells which are quite irregular in size and with cross diameters much exceeding their height except near the upper end of the segment. The tapering is quite uniformly at the rate of 1 to 55. This is the most rapid rate of diminution in diameter shown in any of the portions of sorophores figured.

In the section of sorophore shown in FIGURE 33 the cells again are quite irregular in size and here, as in the upper part of FIGURE 32, we find many of them higher than they are wide. This is characteristic of the terminal region of the slender sorocarps grown in drops of dilute dung decoction, and comes to even more extreme expression in the sorophores shown in FIGURES 34 and 35. Somewhat above the middle of this segment a cell was broken in mounting the preparation. The segment (FIG. 33) tapers at the rate of 1 to 70.

It is obvious in FIGURE 33 as in the previous figures that the

relative vertical space occupied when any two adjacent cells are compared is not alone a matter of their relative position in the sorophore, but also of their actual relative size as produced in the growing and multiplying period. This is shown conspicuously in these terminal segments of the sorophores by the casual way in which the longer and shorter cells are distributed.

The important morphogenetic fact to be noted in these terminal segments is, however, the further striking change in cell form which in these cases the terminal position brings with it. We can note now the culmination of the series of changes in cell form which are involved in the production of the evenly tapering sorophore. At the base we find cells which are approximately isodiametric and take more or less typical least surface forms by mutual pressure. This condition continues in the lower median region. In the upper median region and the lower part of the terminal region we find the cells somewhat flattened, as the number of cells in cross-section is reduced to two or three. This flattening is still more conspicuous higher up in the terminal region. The condition so far might suggest a direct flattening effect on the cells with increased height. But when we come to the upper part of the terminal region we find the relative length of the horizontal and vertical axes of the cells gradually reversing itself and the cells becoming elongated upward.

This condition is very clearly shown in the segment of a sorophore shown in FIGURE 34. I have measured the transverse diameters and heights of the cells beginning with the first cell completely shown at the basal end as number 1. The relation of width to height varies from 1 in width to 6 in height, to 1 in width to 4.6 in height in this series. The average for this single segment of a sorophore is 1 in width to 2.1 in height. I have also measured the widths and heights of the cells in the section of sorophore shown in FIGURE 30, which, as noted, is from a larger sorocarp grown on its natural substratum. This type of sorocarp is illustrated in FIGURE 15. The magnification is very low in this case but an examination of the sorophore just below the sorus shows that the relative widths and heights of its disk-like cells are of the same general order of magnitude as those shown in FIGURE 30. The range in the proportions of these cells is from 1 in width to .3 in height to 1 in width to .7 in height. The average here is about 1 in width to .4 in height. We see that in

the terminal region of the small sorocarp the cells may average over twice as high as they are wide while in the terminal region of the larger sorocarp they may average less than half as high as they are wide. The relations of width to height in all the cells of these two figures are given in TABLE I.

TABLE I *Showing relation of cell width to cell height in segments of two sorophores from their terminal regions. The cells are numbered from the base of the segment upward beginning with the first cell which is fully shown in the figure. FIGURE 30 from a large sorocarp taken from its natural substratum. FIGURE 34 from a smaller sorocarp grown in a hanging drop of horse dung decoction.*

FROM FIGURE 30			FROM FIGURE 34	
Cell	Width	Height	Width	Height
1	1	.6	1	1.4
2	1	.5	1	.8
3	1	.7	1	1.2
4	1	.3	1	.6
5	1	.6	1	1.1
6	1	.3	1	1.1
7	1	.4	1	1.9
8	1	.4	1	1.0
9	1	.5	1	2.4
10	1	.4	1	1.7
11	1	.4	1	1.8
12	1	.6	1	2.2
13	1	.5	1	3.3
14	1	.4	1	2.9
15	1	.5	1	4.1
16	1	.6	1	4.5
17	1	.4	1	4.6
18	1	.5	--	-----
Av.	1	.47	1	2.1

The number of cells measured is not large enough to give an adequate statistical picture of the general situation, but the tendency is unmistakably in the direction indicated. An extended statistical study of these variations would be well worth while, and I am collecting material for it.

The fact that the morphogenetic response of the cells can be reversed so strikingly from the base to the apex of the same sorophore, in the extreme case from proportions of 1 in width to 1 in height, as is common in the basal region of all sorophores, to 1 in width to 4.6 in height (cell 17, FIG. 34) is the point with which we are particularly concerned. We have here the proof that cells, all of the same colony, presumably of about equal age, and probably belonging approximately to the same cell generation from the spore, can show such great and highly adaptive variations in form, depending on the position in which they happen to find themselves in the sorophore. Such morphogenetic responses as this are plainly in the same general category of cell reactions as those which lead to the differences in cell proportions which we find between bast cells and parenchyma, for example, or as any of the other responses affecting cell proportions which we find in the histogenetic development of the higher plants.

FIGURE 35 is from a very poor photograph of the same sorophore shown in FIGURE 34. The rate of tapering in FIGURE 34 is 1 to 61, and in FIGURE 35 it is 1 to 65. The two figures overlap in part. The second entire cell shown at the base of FIGURE 35 is the same as cell 13 counting from the base in FIGURE 34. The relation of width to length becomes even more extreme in the uppermost cells, going as high as 1 in width to 6 in height. The apex itself does not come out in the reproduction, though shown in the negative. The end cell is slightly widened and there is more or less waste material about it such as is also shown about the apex of the sorophore in FIGURE 31.

The section of sorophore in FIGURE 36 is given to show that the protoplasts still have considerable mass in all these young sorophores, though it is not recognizable in the photographs. In this figure the protoplasts are shrunken and their shrivelled masses are conspicuously shown. The cross walls are sometimes obscured by the material deposited on them. The preparation suggests that in fully formed sorophores the cells may be still alive. The rate of tapering shown in FIGURE 36 is 1 to 78.

#### MORPHALLACTIC CURVATURE

I have discussed the morphogenesis of the sorophore from the standpoint of the simplest case, that of a straight, vertically erect tapering column. As a matter of fact, the sorophores are

never absolutely straight but curve variously, all the while maintaining their generally well balanced posture. The most conspicuous case of controlled curve production is that which appears when the sorocarp develops on a sloping or inverted substratum. The sorophore is built out vertically to the substratum for a certain distance in response to negative hygrotopism and then curves upward as a result of negative geotropic response. Such a curvature is not as it is in practically all other cases a so-called growth curvature. The curvature is accomplished by increasing the number of cells on the convex side of the curve or by morphallactic rearrangement of the material of the cells so that they are more or less wedge-shaped with the thinner edge toward the concave side. In such gentle curvatures of the sorophore as are shown in FIGURE 26, it is not at all easy to determine which method or whether both methods have been involved. Such curves are undoubtedly produced at the upper end of the sorophore while the cells are not yet compacted. As noted before, this rounded uncompacted condition of the cells extends (see FIGS. 11 and 13) some distance below the apex of the sorophore and doubtless indicates the limits of the zone in which such curvature adjustments are possible. The evidence for delicacy of response in this case is as noted very striking.

Such regulatory responses for maintaining erectness must be in evidence throughout the development of the sorocarp, since in nature pressure of air currents, as well as slight shifts in the position of the load supported as the decidedly topheavy pseudoplasmodial mass creeps upward, must be continually occurring and require continual slight compensatory shifts in the protoplasmic masses of the cells in the uncompacted zone. Mechanically it would appear that these responses must be, in the case of upright cultures, in the nature of upward thrusts against any increase of the load on a particular flank of the sorophore. The case is similar in this respect to that of functional hypertrophy in growing organs. Here, however, as stated, the sorophore does not increase in height by growth, and the process involves either the shifting of entire cells near the apex, or change in form of the cells, implying a shift of their protoplasmic material. Olive reports also an increase in volume of the cells as they become vacuolated. In either case it is a matter of morphallactic readjustment of mass and the response as in the case of tapering

may be designated as functional morphallaxy to indicate its parallelism with functional hypertrophy. Here again, from the standpoint of morphogenetic theory, the conspicuous fact is that free and individualized cells are able by morphallaxis to duplicate so perfectly the curves and symmetry adjustments which we regard so generally as strictly growth phenomena.

#### BRANCHING SOROCARPS

It has been observed by Brefeld (1884), Olive (1915) and others that *Dictyostelium* occasionally branches. Such a case is shown in FIGURE 5. As Olive has well described, the process consists in simply developing a lateral sorocarp on the main sorophore as a base. The two then form parts of a single symmetrically balanced whole. As the photograph shows, the sorophore of the branch was accidentally broken and bent back upon itself at about three-fourths of its height. The sorus of the branch rests back upon its sorophore in the figure.

The branching of *Dictyostelium* is very simple as compared with the development of the rather evenly spaced verticils of branches of successively diminishing length found in *Polysphondylium*. Olive's studies show that morphogenetically the process is the same in both cases. The branches are duplicates of the main axis in all their structures and are attached to it in the same fashion as it is attached to the general substratum. As noted above, it is not easy to bring out the structure of the base of sorocarps so as to show the basal bulb and its enveloping slime separately because of the density of the mass. The same is true for the origin of branches. Olive's drawings are very clear, and my observations confirm his entirely. In FIGURE 6 is shown with higher magnification the region of origin of the branch of the plant. At this level both the main axis and the branch are shown to be made up respectively of single series of disk-like flattened cells. The branch starts from the main axis at an angle of about  $33^\circ$ , but soon curves in and runs more parallel with it. The branch originates on the upper surface of the curved main axis in a position well adapted for its support. Aside from the accidental position of the sorus of the branch the whole makes a well balanced figure. The sori before the breaking of the branch were rather close together, the sorus of the branch lying somewhat below the plane of the figure. The upper surface

of the base of the branch can be traced almost to the surface of the main axis. Its lower surface is much more obscured by the enveloping slime which forms a broad clasping base partly enveloping the main axis. The curved upper surface of the slime as it fills the axil of the branch gives full expression to the play of forces to which it is subjected in anchoring the branch to the main axis. The basal end of the branch is rounded off and is not moulded in any way to the surface of the main axis. Its attachment to the latter is entirely a matter of the viscosity and distribution of the slime mass. The slime is, of course, entirely inert and its distribution about the base of the branch is due to the rounded form of the mass of amoebae which congregated at the point of origin of the branch. The whole effect is to produce a highly movable and elastic joint. Such a well balanced branching figure illustrates the possibility of producing by morphallactic cell aggregation the same plant habit whose outlines as noted above we associate with and are wont to assume can only be produced as growth curvatures.

#### THE REGULATION OF TAPERING

The work to be done in the morphogenetic processes involved in forming the sorocarp is primarily the lifting of a certain load. That this is not a constant load throughout the process is clear, but, as noted, a number of factors are involved in its variation, whose relative significance is not at once obvious. It might be assumed that at first, while the pseudoplasmodial mass rests largely upon the substratum, the load would be at its minimum. On the other hand, the upward thrust at this stage is against the surface tension of the mound of slime and myxamoebae formed by the aggregation processes. Just how great this is as compared with the weight resistance later is not clear. For a certain period the pseudoplasmodial mass is increased by the addition of further amoebae which creep in from the neighborhood as the figures show. As soon as the sorocarpic mass rises above the surface of the culture medium, there can be no question that a drying-out process begins and is accelerated through all the subsequent stages. It may well be that this progressive drying-out is the most important of all the factors influencing the weight of the mass. Brefeld (1869) emphasized the significance of the slime envelope which covers the whole mass, and speaks of a

point in the development of the stalk when this slime membrane is broken away from the slime remaining at the base of the stalk.

There is no doubt that the weight of the load carried by the developing sorophore at any particular stage is determined by a complex of factors whose adequate analysis will be difficult. It seems to me, however, that at least when once the pseudoplasmodial mass is lifted from the substratum, it will undergo progressive loss in weight by drying and further, comparing the weight of the pseudo-plasmodial mass as a whole at the initiation of sorocarp formation with the weight of the full formed sorus, there can be no doubt that a more or less progressive loss in weight has occurred. We must not forget that those myxamoebae which mould themselves into the tissues of the sorophore as it is formed, thereby remove their own weight from the load to be carried by those above them. Taking all the factors in the situation into account it seems to me it is safe to assume a progressive loss of weight in the load to be carried, especially after the very early stages, and that this diminishing load may be reckoned with as a morphogenetic stimulus.

Perhaps the most notable structural feature of the sorophore as described above and shown in the figures of PLATE 8 is its smoothly and evenly tapering form. We naturally associate this characteristic with an assumption of progressively diminished load to be carried. We have noted that in the erect cultures the production of a vertical column of some sort is provided for by the capacity of the cells to react to stimuli of maximal resistance. This morphogenetic response is basic for the production of the sorophore. The tapering form and gentle curves of the finished product demand further analysis. Taking the whole situation into account it seems to me the factor of relative load carried at different heights is the one to be first considered.

Accepting the conclusions reached above, as to the general result of all the factors affecting the weight of the pseudoplasmodial mass, we may call this the stimulus of diminishing load. This stimulus again, we may assume, operates to induce functional morphallaxies which may effect readjustments in the space relations of entire cells or in the form and distribution of mass of the individual cells. We can not, however, think of such a stimulus as producing a constant alteration in the form of the cells, as, for example, that successive diminution in load



will always produce corresponding increase in the length of the vertical as compared with the transverse axis of a cell. The facts as described above contradict such an assumption. The responses, by which the stimulus of diminishing load controls the tapering form of the sorophore, vary with the height and the general size of the whole sorophore mass. Marked increase of the height as compared with the width of the cells only occurs under certain special conditions described above. The taper in the larger sorophores involves a reduction in the number of cells in the cross-section. That this change can be brought about, not by controlling the rate and plane of cell division, but by the form-determining responses of a group of cells, is the striking morphogenetic fact to be noted. Further, in the analysis of the whole question as to how the general proportions of the sorocarp are determined and the degree of tapering in the sorophore is regulated, we are confronted, as in the case of the coenobic algae, with a sort of symmetry response. There is, it seems to me, evidence that cells will manifest morphallactic readjustment in order to come into symmetrically distributed contact and pressure or tension relations, as far as possible, on all their surfaces. The capacity to respond to the stimuli of maximal resistance and diminishing load, and the functional morphallaxies which give expression to the results of these stimuli, suggest the sensitiveness of the cells to all forms of pressure and contact interrelations. The stimuli of maximal resistance and diminishing load are probably supplemented by these more delicate relations of mutual contact and pressure in the transverse axis of the sorophore. Contact responses may be dependent on stimuli resulting from varying degrees of adhesion between the cells as they dry and ripen out in the maturing of the sorocarp. However brought about, these symmetry responses are very strikingly characteristic of *Dictyostelium*, as well as of morphogenetic phenomena in the higher plants.

#### DISCUSSION

*Pressure and contact stimuli.* If we consider further the question as to the nature of the stimuli concerned in determining the morphogenetic responses of these colonies of myxamoebae, it seems to me that, as in the case of the coenobic algae, we are forced to regard as of great importance these cellular interre-

lations of contact and pressure. The behavior of the myxamoebae in hanging drops shows that neither gravitational nor moisture stimuli are entirely dominant. And it seems probable that the general orientation of the sorocarps to the substratum is determined by the joint effect of negative hygrotopism and negative geotropism. These are the stimuli which direct the creeping upward or downward of the pseudoplasmodium, just as they influence the direction of growth of plants in which growth and morphogenesis go on simultaneously. When we consider the tapering form of the sorophore, its bulbous base and the progressive changes in the relation of height to width in its cells, the stimulus of diminishing load seems to be of primary significance. Curvatures are probably of two general types, those determined by the general hygrotophic and geotropic reactions of the pseudoplasmodium, and those which maintain the general balanced posture of the sorocarp. Both involve morphallactic adjustments in the distribution of entire cells and in the form of individual cells and are achieved in the apical undifferentiated region of the sorophore. The upward movement against gravity is a response to the stimulus of maximal resistance. In downward growth negative hygrotopism would operate similarly through a stimulus of maximal dryness. The parallel in many of these cases with growth responses is striking, but it is to be remembered that here the responses are all achieved by discrete individual cells and that there is no possibility of interpreting them as mass reactions of protoplasm. Primarily such pressure and tension relations are all matters of weight stimuli, the expression of gravitation acting in the field of intercellular relations. The condition of intercellular contacts, partial or complete, may also develop stimuli in connection with the adjustment of the cells to each other as they gradually compact themselves in the sorophore.

The grounds for such assumptions are, it seems to me, fairly clear. The sorophore is primarily a supporting structure. Its proportions of height to width are obviously adapted to the load it has to carry. The building of the sorophore provides for lifting the germ plasm into an advantageous position for drying out and distribution as dustlike spores. The formation of the sorophore involves two morphogenetic elements, the secretion by the amoebae of the inert slime envelope and the aggregation and

deposit successively upon each other of the amoeboid cells. In the simplest cases in which the sorophore consists of only one series of cells, this involves simply the placing of one cell upon another, at first as rounded bodies and later by compacting as disks or shorter or longer tapering cylinders. The outstanding morphogenetic fact is the exactness with which the cells adjust their forms and intercellular contacts so as to produce the evenly tapering outline of the sorophore as a whole. Just how far the secretion of the slime sheath is related to this form regulation is not clear, nor is it entirely clear whether the sheath is a product of the surrounding cells or those in the sorophore itself. In any case the inert slime belongs in the category of cell wall materials and it is a well established fact that it is the protoplast rather than the wall which determines the form of a cell. In the slime sheath, then, or in producing the slime sheath, each cell adjusts itself to those proportions in length and width, which will give just such a reduction in the diameter of the sorophore as will maintain its even taper. When we consider how slight these dimensional changes are, the delicacy of the response is seen to be almost inconceivable.

Furthermore, the cells must make such an adjustment of the distribution of their masses as will develop the curves which the common reaction of all the cells to the environmental stimuli of moisture and gravity require. Doubtless the slime sheath which is secreted serves in some degree to maintain the smoothness of outline of the sorophore, but the morphallactic response by which curves arise must be a matter of direct cell response.

Brefeld (1869, *pl. 3, fig. 21*) figures a case which shows the sheath developed as an empty tube beyond the contained amoeboid cells. I have never seen such a case, and it is not easy to see how it could have arisen, as the whole upper end of the developing sorocarp is a continuous mass of myxamoebae. Brefeld introduced the confusing conception of free cell formation here and his description is not clear. In his first paper, Brefeld (1869) was of the opinion that the amoebae during sorocarp formation fuse to form a true plasmodium, a view which he later corrected (1884). That the sheath once formed aids in support of the sorophore is clear, but as an inert slime its thickness, diameter, etc., must be determined by the cells which secrete it. As noted above, at least in the later stages, it is trumpet-shaped at its

upper end (see FIG. 14) and this condition may persist in less degree in the ripe sorus. That the tapering of the sorophore is not perfectly uniform nor progressively modified from base to apex in any simply mechanical fashion is clear from the figures I have given. None-the-less, in these respects it is quite like axial structures in plants generally. A basal region of rapid tapering is followed by a median region of slight or no tapering and the apical region again tends to taper more rapidly.

In general symmetry of proportions and adaptations for securing spore distribution the sorocarp of *Dictyostelium* compares favorably with *Mucor* or other molds in which the growth and morphogenetic processes are combined as they are in the higher plants.

*Relation to older theories of ontogeny.* As to the bearing which the facts of morphogenesis in *Dictyostelium* have on the older theories of ontogenetic development, it seems to me certain rather definite suggestions are obvious. It is clear that no preformational, mosaic or promorphological representation of the mature sorocarp in the spore is possible. The position of the cells in the sorocarp, their differentiation in form and their ultimate fate as soma or germ plasm are all matters which are determined after the long period of growth and cell division in the free swarming colony is completed. It appears that those amoebae which are earliest to mature or are most vigorous and active in initiating the morphogenetic processes will come to form the sorophore. The remainder of the amoebae will form the sorus and constitute the germ plasm.

For studying the evolutionary differentiation of soma and germ plasm we have, as noted, in the Acrasieae a fairly complete series. There can be no question that this differentiation has been achieved progressively as an adaptive modification, all of whose stages can be assumed to have selection value. As the process works out in ontogeny we are confronted with a striking example of the disregard of the interest of the individuals, here the cells, as compared with those of the species. The very cells which initiate and carry out the process of building the sorophore are sacrificed in the interests of giving the remainder "a better place in the sun." I have pointed out in another connection that the tendency of cells to achieve balanced and symmetrical relations of mutual contact and pressure may be a basic feature

in our appreciation of the aesthetic value of symmetry and balanced proportions. In view of the very obvious suggestion of self determination in many of the cell activities in *Dictyostelium*, it is possible to interpret this action of the myxamoebae in building the stalk on which their fellows may climb to a more advantageous position as a very primitive form of altruistic expression.

That there can be no fixed predisposition of any of the amoebae for any specific part of the sorocarp is well shown by Olive's experiments in which he placed partly developed sorocarps in fresh culture solutions and observed the myxamoebae separate and then renew their morphogenetic activities, by building not one but several new sorocarps, instead of reconstructing the one upon which they were engaged when interrupted by the experiment. These facts of morphogenetic behavior in *Dictyostelium* are to be considered together with the long established fact that certain sponges, when cut up into fragments, creep together and reunite to form new sponges, and the familiar facts of the development of totipotent blastomeres as strong evidence against all mosaic theories of development.

Greil's revival of epigenetic conceptions consists in hardly more than a renewed recognition of the conception that inheriting is achieving. The emphasis thus placed on all forms of morphogenetic stimuli, internal as well as from the external environment, is quite in accord with the evidence for the importance of cellular interactions as shown by *Dictyostelium*. The difficulties in the application of simple epigenetic conceptions to the analysis of the development of the sorocarps in *Dictyostelium* are, however, just as obvious as in the case of more complex forms. In assuming that the cell organization of the myxamoebae and the cormophyte-like organization of the sorocarp are fundamentally dissimilar, I am not assuming any lack of complex organization in the myxamoebae. The specificity of the spores in these simple types is quite as definite as is that of the germ cells in the higher plants. The nature of this specificity is suggested by the character of the reactions by which the sorocarp is built and by the cyclic changes in the reactions of the amoebae at successive stages in their cellular development. What I have called the maturity which marks the end of cell growth and cell division, and is followed by sorocarp formation, may be the result of a cyclic metabolic change such as Child (1915) emphasizes.

His assumption, however, that morphogenetic processes must necessarily be the expression of such changes in growth processes, is not in accord with the facts of sorocarp development. The structure of *Dictyostelium*, and even more that of *Polysphondylium*, with its successive whorls of progressively shortened branches, seems to illustrate very perfectly Child's conception of serially developed structures which owe their differentiation to axial gradients in metabolism. The facts clearly show in this case, however, that just such metameric symmetry in form, with corresponding histogenetic differentiation and variation in prospective function, can all be produced by morphogenetic processes carried out by a colony of cells which grew and divided under similar environmental conditions, and presumptively belong to the same, or almost the same, cell generation. I shall leave the further evidence as to the general organization of the myxamoeba and the relation of the facts of sorocarp formation to Child's theories, to be discussed further in connection with the experimental study of morphogenesis in *Dictyostelium*.

*Organic regulations.* The morphogenetic processes involved in the formation of the sorocarp, as noted earlier, may bring with themselves regulatory stimuli to limit the height of the sorophore. Increase in height of the sorophore carrying the mass of myxamoebae will bring increasing unsteadiness calling for more continuous morphallactic readjustments in the cells of the axial region. When this increase reaches a certain point it may serve practically to prevent the cells from forming the normally compacted type of tissue in the sorophore. This condition may result in inhibitory stimuli which prevent further cells from moving into the apical region of the sorophore.

Another variable which may afford organic regulation in respect to height is found in the relation of weight carried to the cross section of the sorophore. A remote approach, even, to the crushing point for its cells may serve to prevent the addition of further cells to the sorophore. Whether or not the height of the sorophore is regulated by these particular factors in the situation, the possibility of the internal production of organic regulations in such a process is strongly suggested. Given a system of plastic cell units capable of responding to stimuli which lead to morphallactic readjustments in the distribution of their inter- and intra-cellular mass relations, whose function it is to raise a

shifting and unsteady load to a height well within the crushing limits of the system, the intercellular pressure relations may be conceived as providing the stimuli which will determine the height to be reached.

*Organization of the cell of the metaphyte.* Lundegårdh (1914) in one of the more recent attempts at a statement of a so-called chemo-physical theory of life phenomena, makes as a basic working hypothesis the assumption that there is in principle no difference between a unicellular and a multicellular organism since the latter is merely "a heap of cells," all of which have the same makeup, and this makeup is chemo-physical. This hypothesis should be capable of clear illustration in the case of *Dictyostelium*, since the sorocarp is visibly in its origin "a heap of cells." But the evidence that the organization of the sorocarp is the same as the organization of one of the myxamoebae that built it is certainly not obvious. Such a statement puts an extreme emphasis on the conception of the individual, whether one- or many-celled, which it seems to me is opposed to all the fundamental facts which have come to constitute the cell theory. Lundegårdh everywhere emphasizes the conception of the cell as a mechanism, but in claiming the equivalence of one and many-celled organisms he does not sufficiently recognize the fact that, in simple phrase, machines are not aggregates of smaller machines of the same kind.

I have elsewhere stated the grounds for regarding protophytes and metaphytes as incommensurables from the standpoint of their organization; and the facts as to the structure and life histories of such forms as *Dictyostelium* and *Polysphondylium* are, it seems to me, further evidence for the correctness of this assumption. To compare the erect radial and metameric plan of organization of *Polysphondylium* with the amoeboid form and protoplasmic constitution of one of the cells which helped to build it, is to overlook, as Lundegårdh does, the essential and obvious facts of observation in the interests of a generalization so broad as to have lost all important concrete content.

The specificity of many-celled organisms is in general most obvious at least in their form characters, and it is the *morphogenetic* predispositions and capacities of the cells that are most in need of analysis. In *Dictyostelium* it is to be noted that all of the morphogenetic cell capacities which we have postulated

involve the functioning of the cell body as a whole. The morphogenetic processes by which the sorocarp is organized out of the discrete myxamoebae are matters of cell responses in which the cell behaves as a unit organism. The sorocarp is an organized aggregate of myxamoebae, but the myxamoeba is a biologic unit and not an aggregate of organisms.

I am aware that my analysis of the complex processes of form differentiation in *Dictyostelium* is incomplete and on many points inadequate, but that these transition types between protophytes and metaphytes present the same problems of morphogenesis, though in a simpler form than that in which they are presented in the higher plants, it seems to me can hardly be questioned.

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### Explanation of plates

All the figures are of *Dictyostelium mucoroides* Bref., and the photographs were made with the Zeiss apochromatic objectives 8 mm. and 16 mm., and compensating eye pieces, except FIGURES 1 to 5 and 16, which were made with the Zeiss microplanars.

#### Plate 6

FIG. 1. Entire sorocarp in hanging drop culture photographed from above, showing that the sorophore, after being built downward for a short distance under the influence of negative hygrotopism, curves into a horizontal direction as a response to the joint stimuli of negative hygrotopism and negative geotropism.  $\times$  about 20.

FIG. 2. Two entire sorocarps with curving sorophores grown and photographed as in the case of FIG. 1.  $\times$  about 10.

FIG. 3. Sorocarp with very short sorophore grown and photographed as in the case of FIG. 1.  $\times$  about 10.

FIG. 4. Sorus photographed as in the case of FIG. 1 to show globular form.  $\times$  about 60.

FIG. 5. Branching sorocarp photographed as in the case of FIG. 1.  $\times$  about 10.

FIG. 6. More highly magnified view of region of connection between branch and main axis.  $\times$  about 50.

FIG. 7. Slowly moving myxamoebae in stage of growth and multiplication with many bacteria in culture medium.  $\times$  about 300.

FIGS. 8, 9, 10. Actively creeping myxamoebae from three different cultures.  $\times$  about 300.

FIG. 11. Portion of sorophore with pseudoplasmodial strand ascending on it.

FIG. 12. Portion of same sorophore higher up, where the pseudoplasmodial strand expands into the apical mass.

FIG. 13. Portion of terminal pseudoplasmodial mass showing the sorophore in its median region.

FIG. 14. Apex of pseudoplasmodial mass showing trumpet shaped form of the upper end of the sorophore.

FIG. 15. Portion of sorophore and sorus with spores spread out in thin film.

FIG. 16. Portion of culture with myxamoebae forming a continuous nodular film. The lower right hand corner of the figure extends into the marginal region of the culture, where the amoebae had accumulated to form a still thicker corrugated zone which extended around the entire culture. In a diagonal line a little above the middle of the figure are shown two rudiments of sorocarps between which the myxamoebae are creeping back and forth.

#### Plate 7

FIG. 17. Very young aggregation stage. The young sorocarp, is forming in a very faintly shown projection of the margin of the culture drop in the upper part of the picture.  $\times$  about 300.

FIGS. 18, 19, 20 and 21. Show successive early stages in the formation of the same sorocarp. The margin of the culture drop in each case is shown as a clean cut more or less perfect arc of a circle extending across the figures well above their

middle regions. The myxamoebae push out the margin of the culture medium, apparently as a negative hygrotopic reaction. They are shown creeping towards the sorocarpic aggregations singly and in rudimentary pseudoplasmodial strands.  $\times$  about 250.

FIG. 22. Shows a young sorocarpic aggregation with single myxamoebae creeping toward it, primarily from the marginal region to the left.  $\times$  about 250.

FIG. 23. Shows a young sorocarp whose development has been checked. Most of the myxamoebae in or near the sorocarpic aggregation have separated slightly from each other and are more or less rounded up.  $\times$  about 250.

### Plate 8

FIG. 24. Portion of sorophore from base of a large sorocarp. Cells forming parenchymatous tissue.  $\times$  about 1000.

FIG. 25. Portion of sorophore from median region. Taper 1 to 112.  $\times$  about 1000.

FIG. 26. Portion of sorophore from median region showing a rather abrupt narrowing near its upper end. Taper from base to the region of abrupt narrowing 1 to 103. Taper for whole length 1 to 48.  $\times$  about 1000.

FIG. 27. Portion of sorophore from median region showing rather uneven outlines.  $\times$  about 1000.

FIG. 28. Portion of sorophore from median region. No measurable taper.  $\times$  about 400.

FIG. 29. Portion of sorophore from terminal region. Taper 1 to 67.  $\times$  about 1000.

FIG. 30. Portion of sorophore from terminal region. No measurable taper, but the middle region slightly narrowed, perhaps because of the loss of a cell.  $\times$  about 1000.

FIG. 31. Portion of sorophore showing its apex as it ends in the sorus. Outlines rather uneven.  $\times$  about 1000.

FIG. 32. Portion of sorophore from terminal region. Taper 1 to 55.  $\times$  about 500.

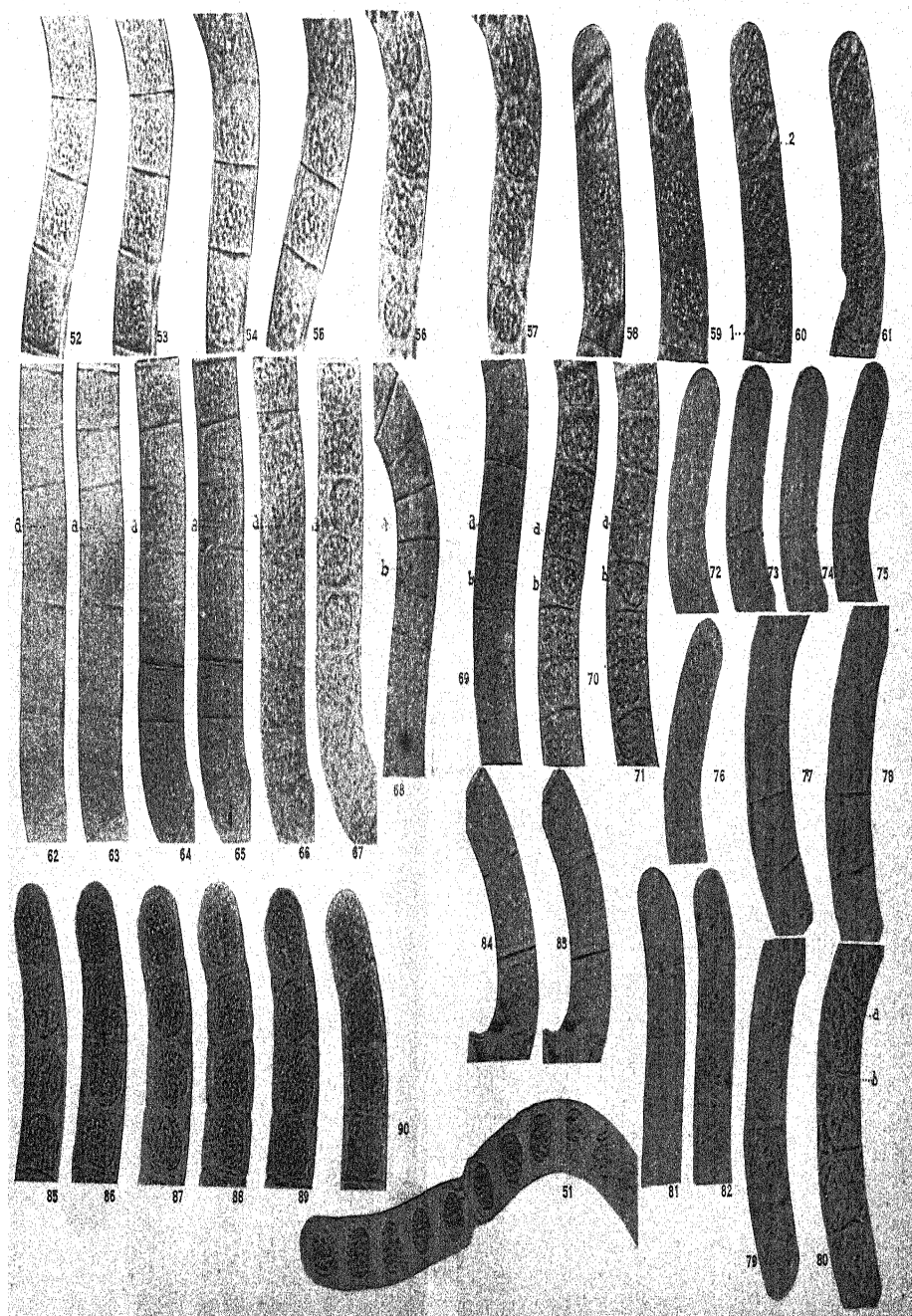
FIG. 33. Portion of sorophore from terminal region. Taper 1 to 70.  $\times$  about 500.

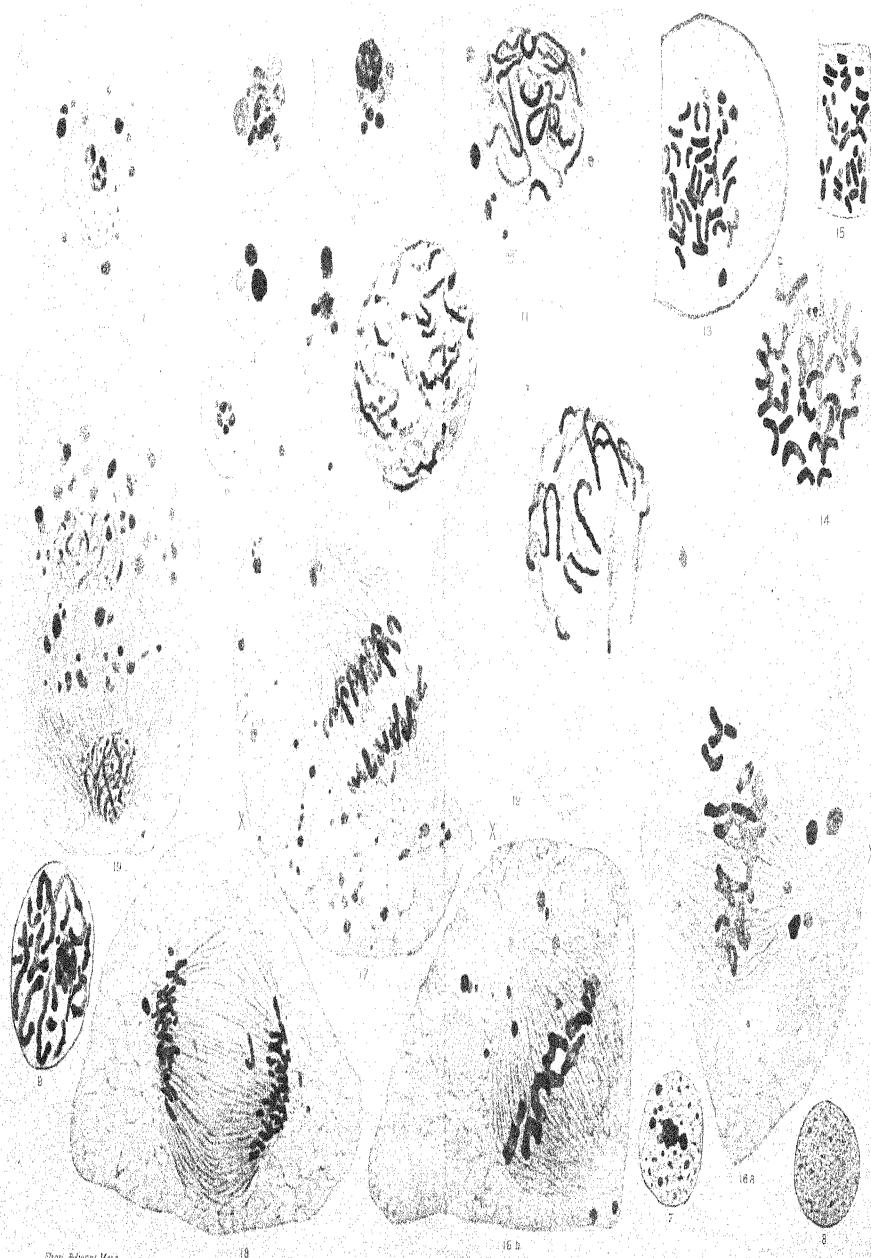
FIG. 34. Portion of sorophore from terminal region. Many cells elongated upward. Taper 1 to 61.  $\times$  about 500.

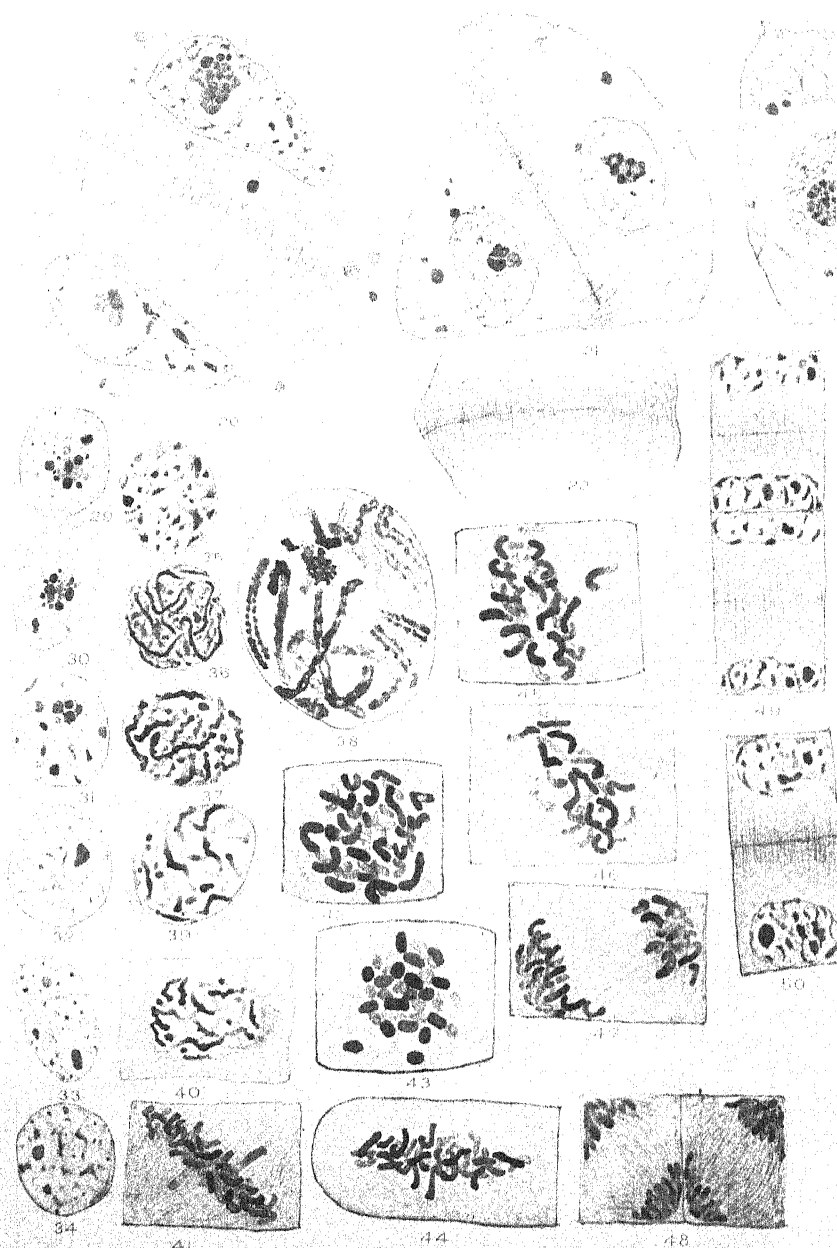
FIG. 35. Apical region of same sorophore as shown in FIG. 34.  $\times$  about 500.

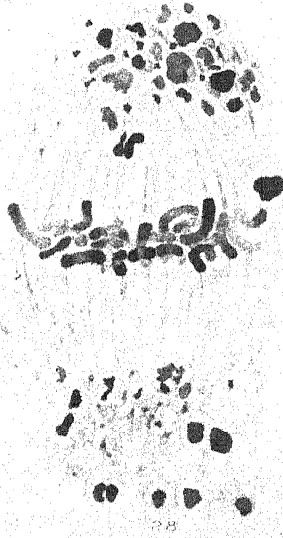
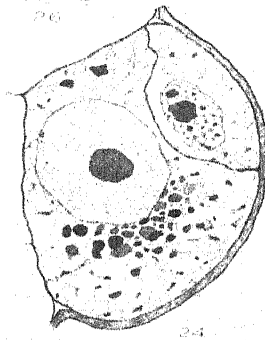
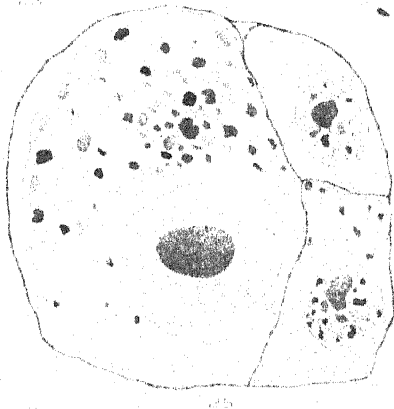
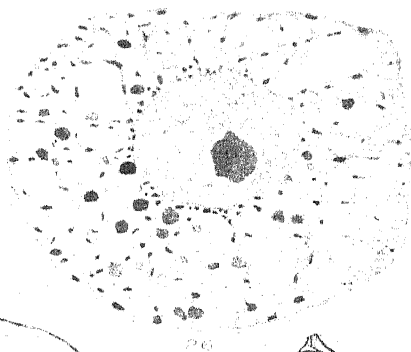
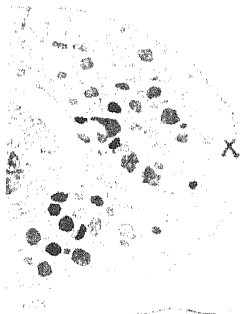
FIG. 36. Portion of sorophore from terminal region. Protoplasts collapsed and shrivelled. Taper 1 to 78.  $\times$  about 500.





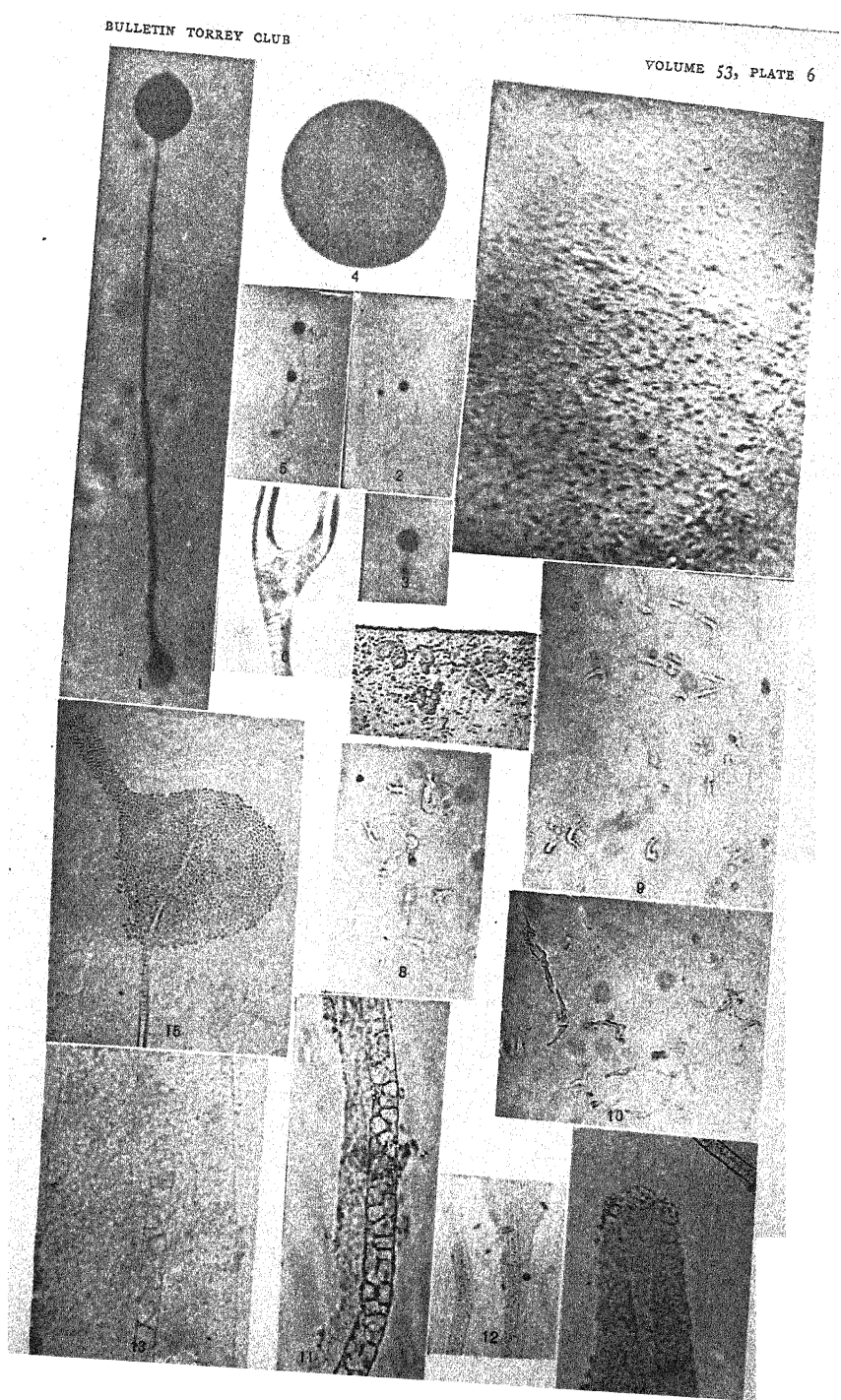




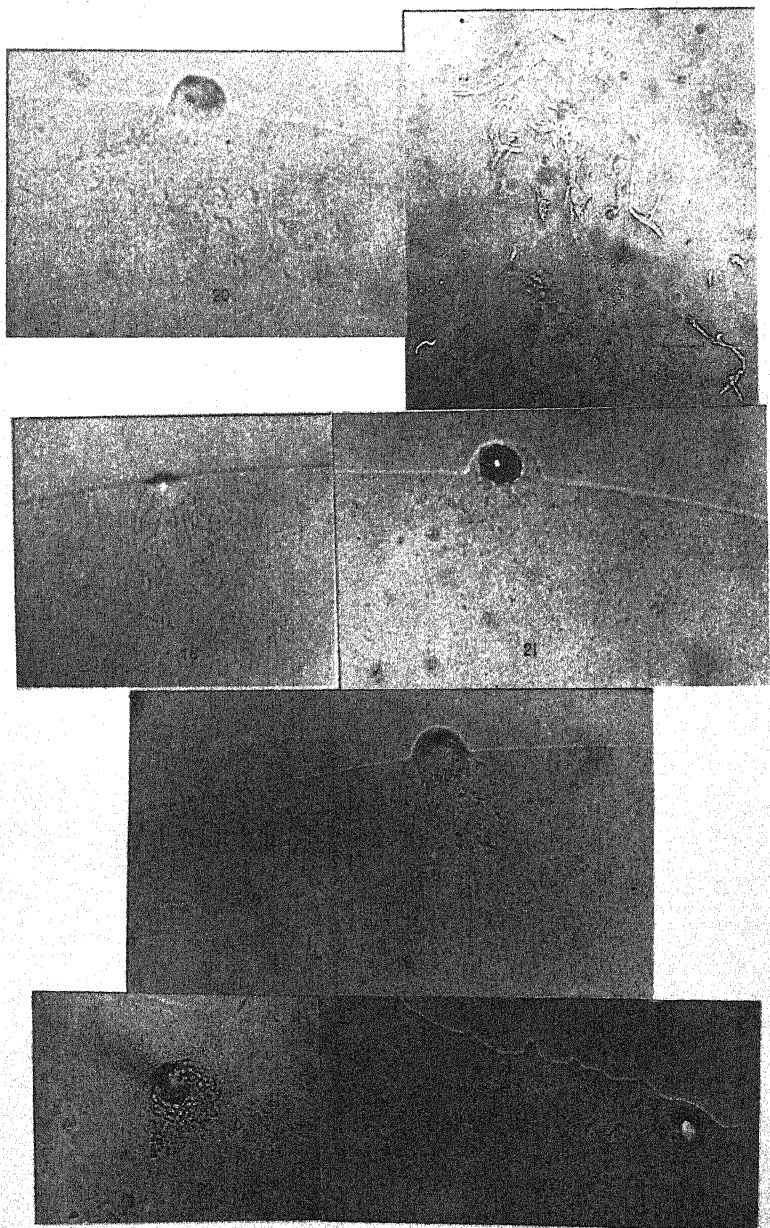


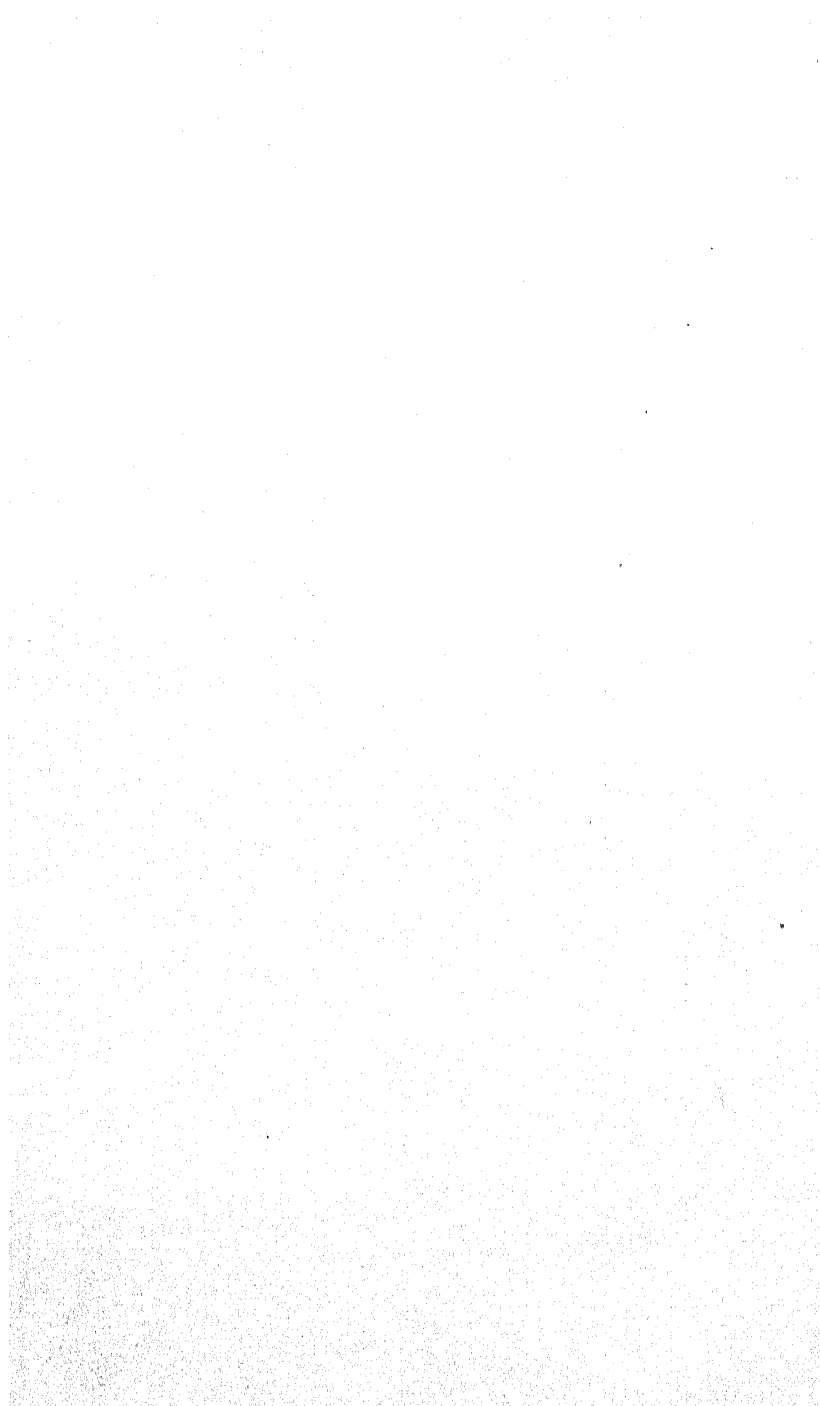


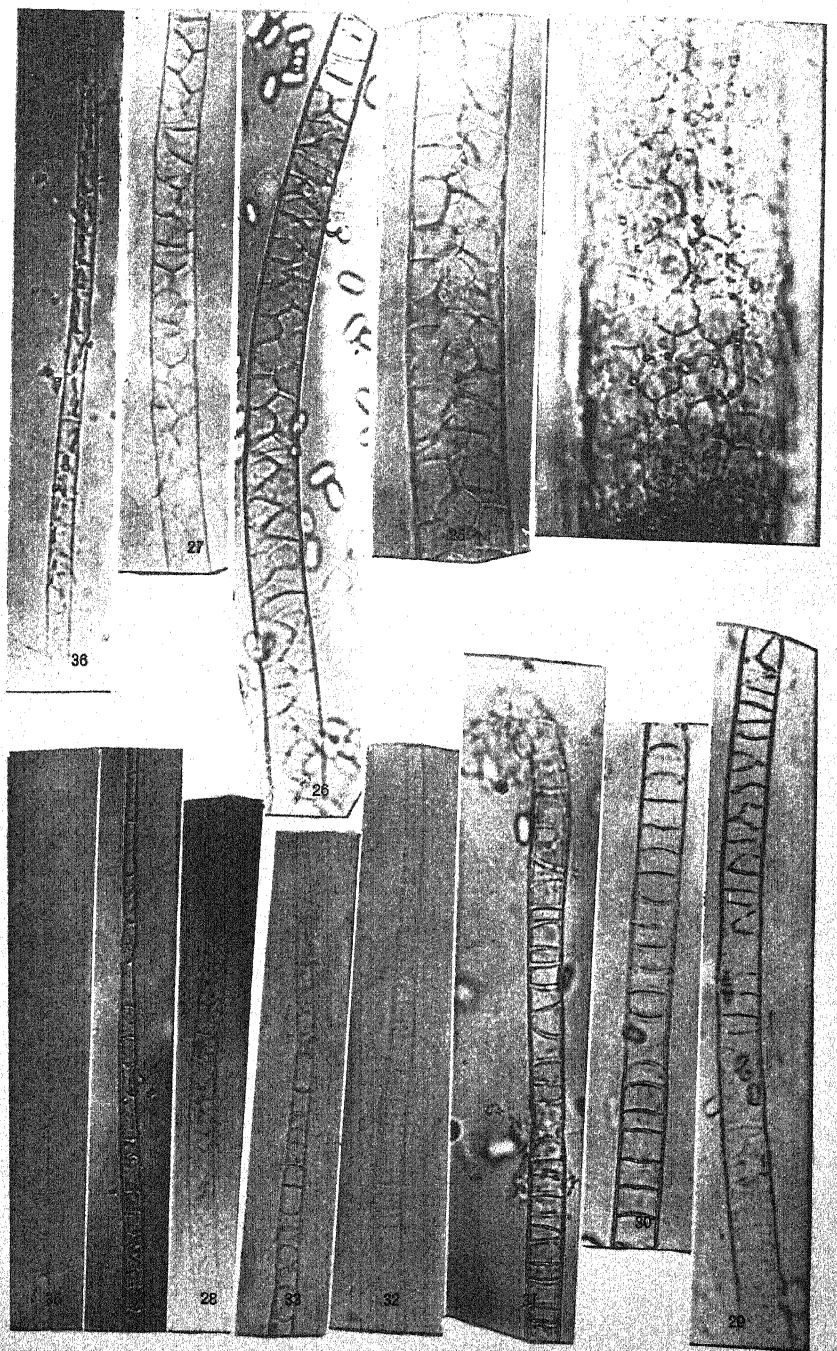














The capsules, seed, and seedlings of the tiger lily,  
*Lilium tigrinum*

A. B. STOUT

(WITH FOUR TEXT FIGURES)

For at least fifty years, tiger lilies have been rather widely cultivated in European and American gardens. The bulbs of this lily first came into England as early as 1804, and there have been many shipments of the bulbs of tiger lilies to Europe and America from the Orient, and especially during the last fifty years of trade relations. At least five varieties differing in some particular from the general type of the tiger lily have come into culture.

As far as the writer has been able to determine, there is no record that any of the tiger lilies have ever been self-fruitful. There is oft repeated mention that they have been entirely fruitless. There seems to be no record, at least outside of Japanese or Chinese literature, as to how these varieties originated—whether from seeds or as bud sports. Apparently the propagation of all the types of tiger lilies has been entirely vegetative by means of the divisions of the mother bulbs and the use of the bulblets which are abundantly produced as buds along the stems in the axils of the leaves.

In the Orient the tiger lily has been in cultivation, it is said, for more than a thousand years. There it evidently exists in cultivation and as an escape far beyond its original habitat. Mr. Ernest H. Wilson, who speaks from much personal observation in the Orient, states of this lily in his recent book "The Lilies of Eastern Asia,"

In China I have seen it undoubtedly wild on the foothills of the Lushan range in Kiangsi province, but nowhere else. In western Hupeh and in Szech'uan I often met with it apparently wild, but close investigation always proved that it has escaped. I believe that it is indigenous on the mountains of Chekiang and Kiangsu provinces in eastern China, and regard the Lushan range as marking the western limit of its distribution.

But Mr. Wilson has never seen capsules on this lily in the Orient. In a letter to the writer dated January 29, 1926, he makes the following statement:—"During my travels in the Far East I never saw *Lilium tigrinum* bearing fruit." Evi-

dently no person has seen such capsules, for all persons who have discussed the lilies of the Orient, and some of these have travelled rather extensively in those lands, repeatedly state that the capsules of the tiger lily were to them unknown.

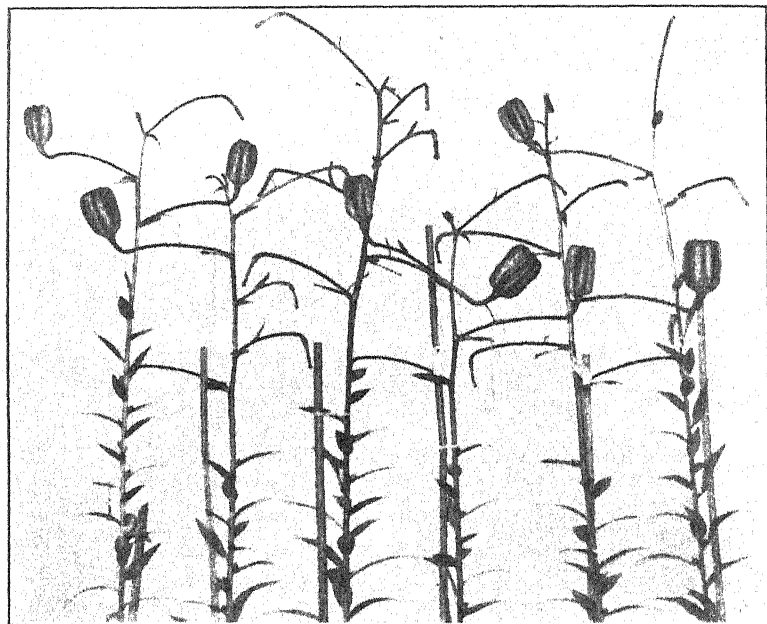


FIG. 1. When there is proper cross-pollination with certain other types of lilies, tiger lilies will produce such capsules as are shown above. This opportunity almost never occurs as the plants are grown in gardens. When the flowers are self-pollinated, or when pollination is from flower to flower on different tiger lilies, capsules do not even start to develop. But sister flowers properly cross-pollinated yield large capsules. The tiger lilies have not lost the ability to bear fruit and seeds because of vegetative propagation. The capsules here shown are from pollen of *L. sutchuenense*, and were obtained in 1923 at the New York Botanical Garden.

THE TIGER LILY IS HIGHLY FRUITFUL IN CROSSES WITH CERTAIN  
OTHER KINDS OF LILIES

Except for the plants of the *flore-pleno* type, which have aborted pistils, all plants of tiger lilies are highly fruitful in crosses with certain rather closely related species. At the New



York Botanical Garden plants of the tiger lily have yielded capsules and viable seeds to the pollen of four different kinds (species?) of lilies.

To the pollen of *Lilium Maximowiczii*, and *Lilium sutchuenense* about 100 fine capsules were obtained which yielded several

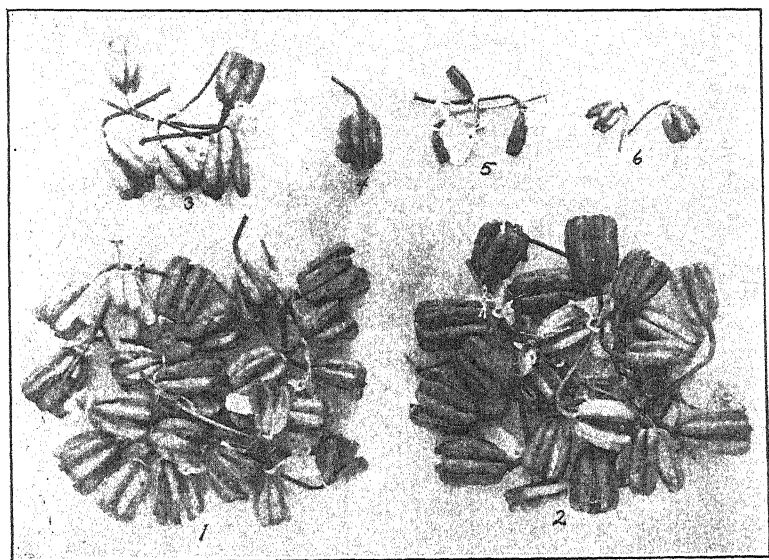


FIG. 2. At 1 above are shown capsules of the tiger lilies obtained from pollination with *L. sutchuenense*, and those at 2 are from pollen of *L. Maximowiczii*. Those two crosses give such capsules as these for nearly every flower pollinated. At 3 are shown some of the capsules obtained from the cross *L. tigrinum*  $\times$  *L. Leichtlinii*; at 4, one of the capsules resulting from the use of pollen of *L. davuricum Wallacei*. Thus far in the experiments at the New York Botanical Garden tiger lilies have yielded only such immature and seedless capsules as are shown at 5 to pollen of *L. warleyense*. At 6 are shown capsules obtained on *L. warleyense* when the pollen of a tiger lily was used in controlled pollination.

thousand viable seeds. Some of these capsules were shown (Stout, 1922, 1923) in what appear to be the first illustrations ever published of an authentic capsule of the tiger lily. The two species just mentioned closely resemble the tiger lily in general appearance, and the ease with which they cross with it suggests a close kinship.

These results clearly demonstrate that the flowers of the tiger lily are fully able to function in producing fruit. The plants have not lost their ability to yield fruit and viable seeds. They merely require a proper cross-pollination with certain other types. When this is done, anyone may obtain seeds and grow the hybrid seedlings.

Capsules with viable seeds may also be secured in other crosses. To pollen of *Lilium Leichtlinii*, twenty-six flowers of the tiger lily gave ten good capsules containing many viable seeds and sixteen partly developed capsules. Of thirteen flowers given pollen of *L. davuricum Wallacei* two were complete failures, nine yielded partially developed capsules, and two matured capsules with a few viable seeds. The species *L. Leichtlinii* is obviously rather closely related to the tiger lily. Although the variety *Wallacei* is listed as a variety of *L. davuricum* or *L. elegans* it has a flower of rather striking resemblance to that of the tiger lily. The tiger lilies do not set fruit so readily in these crosses as they do with *L. Maximowiczii* and *L. sutchuenense*. One experiences a greater number of failures. The requirements for fertilization are more exacting and less compatible.

The seeds obtained in all these crosses have been planted and the seedlings which survive will be grown to maturity. Thus far only one has bloomed. It has the *L. sutchuenense* as a pollen parent. This plant does not have bulbils in the axils of the leaves. Its flowers somewhat resemble those of the tiger but differ slightly in color and in spotting (see FIG. 4).

To pollen of several other species, the tiger lilies have given mostly complete failures and only occasionally a poorly matured capsule. Thus far only such results have been obtained when pollen of *L. warleyense*, *L. pseudotigrinum*, and *L. Batemanniae* was used. Of all the lilies thus far studied by the writer those of *L. Leichtlinii* and *L. pseudotigrinum* most closely resemble *L. tigrinum*, yet of these two only the former has successfully crossed with it.

The true identity of the plants obtained under these species names is a matter on which the writer does not wish to attempt a final opinion. Mr. Wilson in "The Lilies of Eastern Asia" does not recognize some of them as good species. The plants which the writer obtained under these different names were clearly of somewhat different and distinct types. If not good species they were at least varieties.

In the pollinations thus far made at the New York Botanical Garden, capsules have not even started to form on tiger lilies to cross-pollination with the species *auratum*, *canadense*, *candidum*, *chalcedonicum*, many varieties of *davuricum*, *Henryi*, *Humboldtii*, *Roezlii*, *speciosum*, *superbum* and *umbellatum*.

OTHER RECORDS OF CAPSULES PRODUCED BY PLANTS OF  
LILIUM TIGRINUM

The cross *L. tigrinum*  $\times$  *L. Maximowiczii* has also been successfully made by Miss Isabel Preston (1924, 1925 and 1926).

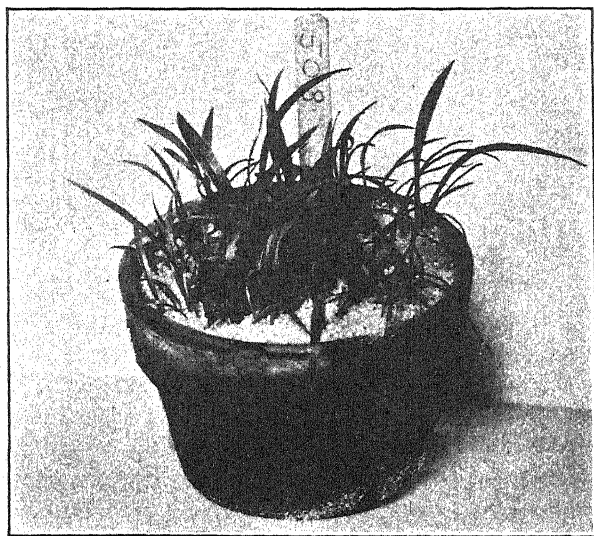


FIG. 3. Lusty seedlings may be had from the hybrid seeds obtained on tiger lilies. Those here shown have *L. Leichtlinii* as the pollen parent.

Her first seed to this cross was secured in 1921 but this was not reported in print or known to the writer until after his publications of 1922. Miss Preston illustrates a flower of this hybrid, which is evidently similar to the one here shown in FIG. 4.

Miss Preston also reports having obtained seeds to the cross *L. tigrinum*  $\times$  *L. warleyense* (*L. Willmottiae*) and she shows a photograph of the hybrid in flower. This cross has repeatedly failed at the New York Botanical Garden.

Mention is made by Miss Preston of seedlings of *Lilium speciosum*  $\times$  *L. tigrinum* produced at the Ontario Agricultural College, which, however, never grew to flowering size.

In a report of certain hybridizations made about twenty years ago in Australia (Kerslake, 1906) there is the mere statement that the cross "*L. tigrinum*  $\times$  *L. elegans Wallacei* resulted in every flower operated upon producing huge pods of seed." This cross is one of those which has succeeded at the New York Botanical Garden.

In his beautifully illustrated monograph on the genus *Lilium*, Elwes (1880) makes the following statement in reference to *Lilium tigrinum*:—

Everywhere in China and Japan it is cultivated and the bulbs are eaten by the natives: but I never saw the capsules and seeds though they are figured by Nees von Esenbeck, 'Genera Plantarum,' vol. II. Mr. Hanson, of New York, informs me that he has been successful in raising many seedlings from this plant, some of which differed remarkably from the parent, both in the form and color of the leaves and flowers: but, owing to a fire which destroyed the whole of these seedlings, I am unable to describe them more particularly.

Mr. Hanson says that to induce the plant to seed, all the bulblets must be removed, and that the seeds, if sown at once in a frame, germinate quickly and produce flowering plants in three or four years.

It may be said that the capsules figured by Nees von Esenbeck and labelled as those of "*Lilii tigrini*" are included in a plate with flowers, flower parts, and a bulb of *Lilium Martagon* to illustrate the various parts of a typical lily. The capsules are longer and of a somewhat different shape than those the writer here illustrates. It is perhaps doubtful that the capsules drawn for the plate in Genera Plantarum came from a plant of *Lilium tigrinum*.

The statements of Elwes, quoted above, make it clear that Mr. Hanson obtained seed from plants of the tiger lily and grew the seedlings. But Mr. Hanson is in error in considering that the removal of bulblets axillary to the leaves led to the production of the seed he obtained. Evidently he was merely reflecting a rather popular view which has survived even to the present time. Mr. Hanson had, it is stated by Elwes, "one of the finest collections of lilies in the world." Without a doubt he grew plants of *Lilium tigrinum* by the side of such species as *L. Maximowiczii* and the insects made the cross-pollinations which were responsible for the capsules which he obtained from his

tiger lilies. That he obtained fruit when he removed bulbils was merely a coincidence. Had he enclosed the flowers of such plants in paper bags and prevented all pollinations except selfings his plants would have been fruitless.

It is really surprising that results such as Mr. Hanson reported have not been observed rather frequently. While the species named above which readily cross with the tiger lily are rarely

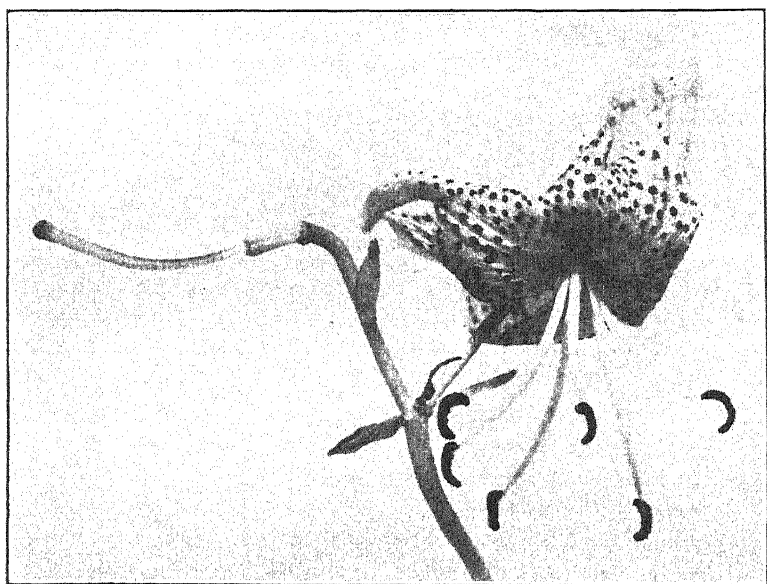


FIG. 4. Flower of a hybrid lily having the tiger lily as its seed parent and *L. sutchuenense* as its pollen parent. The plant does not bear bulbils on the stem and the color of the flower and the spotting are slightly different from the seed parent.

seen in gardens, it is to be expected that they may be grown along with *Lilium tigrinum* in the gardens of fanciers of lilies and in nurseries concerned with producing lily bulbs for the trade. Especially may this be expected to occur in the Orient.

Evidently at least one such hybrid has appeared. In an English garden magazine *The Florist* for 1873, the flower of a lily was illustrated in color, and described as *Lilium tigrinum* variety *Lishmanni*. It differed from the tiger lilies in having no

bulbils, in the smaller size of the flowers and in somewhat different color and spotting. Evidently this plant was very similar to that which the writer shows in FIG. 4 accompanying this article.

One type of lily cultivated by the Chinese in Yunnan has been considered as a hybrid between *L. tigrinum* and *L. tenuifolium*. But Mr. Wilson in his recent book already mentioned assigns this plant to the species *L. Davidii*.

Possibly other hybrids, seedlings of *L. tigrinum*, have appeared in the Orient and some may still be in existence there. The readiness with which they may be obtained in experimental breeding makes this quite probable.

#### THE TIGER LILY AS A POLLEN PARENT

The pollen of the tiger lilies is abundant and is highly viable in artificial culture. A high percentage of the grains germinate and there is a vigorous growth of the pollen tubes. The pollen is excellent. There has been little opportunity to use this pollen in the reciprocals of those crosses that yield fruit on *Lilium tigrinum*. Only four flowers of *L. Maximowiczii* were thus cross-pollinated and these were complete failures. But when the pollen of the tiger lily was used in guarded and controlled pollination on four flowers of *Lilium warleyense*, three fine capsules and some viable seeds were obtained. This result is sufficient to show that the pollen of the tiger lily is able to function in certain relations.

Miss Preston reports that she obtained capsules and seed from flowers of *L. speciosum* pollinated from the tiger lily but that the seeds did not germinate. This cross has always failed at the New York Botanical Garden.

The writer has thus far found no other references to the experimental use of *L. tigrinum* as a pollen parent in breeding work.

#### THE TIGER LILIES ARE ENTIRELY FRUITLESS TO ALL POLLINATION AMONG THEMSELVES

During the past ten years more than 200 bulbs of the tiger lilies have been secured from various sources for use in experimental studies at the New York Botanical Garden. These include bulbs of the type most usually seen in cultivation, of the varieties *Fortunei*, *splendens*, and *flore-pleno*, and also of plants

apparently wild at Kuling, obtained by Dean J. L. Buck, of the University of Nanking, China. About half of these various lilies have lived to bloom. Many pollinations were made, both of individual flowers and from flower to flower on different plants. In no case did a capsule even start to develop. In the first years of study the bulblets were not allowed to develop in the axils of leaves for a number of the plants. In these tests the entire race of tiger lilies has been entirely self- and intra-sterile. This behavior is quite in agreement with the many reports that the tiger lilies have been fruitless. The results of the experimental studies confirm the observations of the gardeners.

#### IS THE TIGER LILY A GOOD SPECIES OR MERELY A CLONAL VARIETY?

Since the tiger lilies do not produce seeds to any sort of pollination among themselves there is no seed that can be used to propagate their kind. They yield seeds only to cross-pollination with different types of lilies and this gives hybrids different from the tiger parent. The evidence indicates that the tiger lilies have always been propagated exclusively from daughter bulbs and the stem bulblets. If this be the case, then the tiger lilies do not constitute a good species breeding true from seed, but merely a clonal strain or variety.

Such a variety has its beginning as a single seedling which is ever afterward propagated vegetatively. In the case of the tiger lily this seedling may have been a hybrid or it may have been a variant from some other type of lily now extinct or perhaps considered as a distinct species.

There is, however, a possibility that the tiger lilies do not constitute a single clonal group and that further search may discover different clonal strains that are cross-compatible. A collection of tiger lilies from widely separated localities in Japan and China would allow one to make more satisfactory tests for this than have thus far been possible. It is even possible that there are localities in China where tiger lilies are really wild and propagating by seed.

#### THE TYPE OF STERILITY IN THE TIGER LILIES

The flowers of the tiger lilies, excepting only the double flowered form with its aborted pistils, are perfect. The pistils are able to develop into capsules. The pollen is highly viable

and will function in certain relations. The plants are fully able to mature at the same time both fruits with viable seeds and bulbs and bulblets. They are self-fruitless because the essential organs—the pistils with their ovules and eggs and the pollen tubes and their sperms—do not react in the manner necessary for fertilization. There is, one may say, a physiological incompatibility in the processes of fertilization necessary for seed formation.

This type of sterility is indeed very common among plants, both wild and cultivated, that have perfect flowers. It exists among annuals that are propagated only by seeds. It is present in plants that are propagated vegetatively and in this case the plants of the same clon will not “cross,” for the pollination between plants is not crossing but is in reality only the same as pollinating from flower to flower on one plant.

Sterility from self-incompatibility is the rule in all of the thirty odd species of lilies tested at the New York Botanical Garden. Even in species that commonly yield seed abundantly (*L. regale*, *L. longiflorum*, *L. Henryi*, *L. speciosum*, *L. superbum*, *L. tenuifolium*, etc.) many seedling plants are as completely self-incompatible as is the tiger lily. But in these the species includes numerous clonal strains and is grown from seed rather generally. Although there is also some cross-incompatibility between plants known to be different seedlings, there are usually, in a planting of these lilies, enough different strains to provide for compatible crossings. Abundant capsules and seed on the lilies in one's garden almost always mean that there are different seedlings or clons present and that the insects have made cross-pollinations between them.

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# Abnormalities in *Botrychium* and certain other ferns

M. A. CHRYSLER

(WITH FIVE TEXT FIGURES AND PLATE 9)

Abnormalities in the spore-producing members in *Botrychium* have been frequently reported, and many unusual specimens are to be found in the larger herbaria, yet a comprehensive analysis of the variations has apparently not been made. Taxonomic writers have for the most part contented themselves with a mere mention of the occurrence of abnormal spikes (Pretz, 14) or with a list of the forms encountered in a specific area (Batchelder, 2). Luerssen (12) however, has grouped the "monstrosities" of *Botrychium Lunaria* in twenty-two classes, and his three main groups will be followed here. In the present paper it is proposed to offer some analysis of the writer's observations of the genus *Botrychium* in field and herbarium, and to show the bearing of aberrant forms in other ferns upon the interpretation which is offered.

The following classes of abnormalities may be distinguished:

1. Branching or duplication of the fertile spike, as a result of:  
(a) Splitting or choris. (b) Wide separation of an ordinary branch. (c) Reversion.
2. Occurrence of sporangia on pinnae ordinarily sterile.
3. More or less complete sterilization of the fertile spike.

1. With respect to forked or otherwise divided fertile spikes, the writer (8) has already committed himself to the view that specimens of *B. obliquum* Muhl, *et al.*, having a pair of spikes in place of the single one, represent a reappearance of an ancestral condition, and from this view he sees no reason to recede, but it does not appear that certain of the forked specimens found in other divisions of the genus can be thus interpreted. The doubt in these cases arose from the fact that in forking specimens of *B. virginianum* (L.) Sw. the two branches are generally unequal. In May 1924, an opportunity was afforded for examining the vascular supply of a forking specimen, for two of these interesting plants were found by the writer near New Brunswick, New Jersey. Each of these examples was a vigorous plant two feet high, and had a fertile spike which forked rather unequally at a point half way up the stalk. The forking region of one plant

was cut into serial sections, and the vascular strands traced. It has been shown (8) that the vascular system of a normal spike consists of a right and a left hand strand, derived respectively from points near the right and left edges of the C-shaped vascular strand of the petiole. In our forking spike there was the usual pair of strands below the level of the fork, each strand being more or less broken up, as is frequently the case in this species. Where the spike forked a portion of the left-hand strand accompanied the right-hand strand into the right fork, leaving somewhat less than the left-hand half to supply the left fork of the fertile spike, which fork was the more slender of the two, as might be expected. This procedure is in marked contrast to the state of affairs in specimens of *B. obliquum* possessing a double spike, for in these the right-hand strand supplies one spike and the left-hand strand the other spike. This difference in the vascular supply in forking specimens belonging to two different divisions of the genus led the writer to suspect that there are at least two independent phenomena which should be distinguished. With this idea in mind he has recently examined all of the specimens of *Botrychium* which were readily available, and has found an unexpectedly large number of cases of branching spikes, amounting to over 170, located in the Academy of Natural Sciences of Philadelphia, Brooklyn Botanic Garden, New York Botanical Garden, University of Pennsylvania, Yale University, United States National Museum, and in several smaller collections. As the study proceeded it became evident that practically all of the branched spikes in *B. virginianum* were divided unequally, generally very definitely so. The location of the fork may be at almost any level, from near the base of the fertile spike to near the spore-bearing portion. The abnormality may occur in large specimens and also in depauperate individuals, and is represented by plants from a wide range (New England, Japan, Ecuador for instance). Dr. Kelley has called my attention to a large forking specimen in his collection which had been damaged by a falling branch. Although most specimens show no evidence of wounding, such stimulus would probably have to be exerted in the preceding year upon the embryonic leaf, and hence would not show in the mature leaf except in the form of such abnormality as has been described. It is at any rate submitted that such stimulus as a wound may bring about a splitting of the apical

region of a young leaf, a phenomenon referred to by Bower (4) as chorisis, and by him in his earlier works regarded as the only significance of branched spikes, wherever they may occur. It may be remarked in passing that certain cases of branching of the spike in *Ophioglossum* (Bower, 5) are readily explained on the basis of splitting, and in one such case the anatomy of the leaf has been worked out (Holden, 11) and found to be in accord with such a view.

There appears to be another group of cases of forked or divided spikes which can be most readily interpreted as branches of the regular monopodial order which are unusually remote from the other (higher) branches of the spike. The branches of a fertile spike are often distinctly alternate, and it can be readily seen how a strong basal branch may diverge at an unusually low level and give the appearance of an unequal fork. A large number of cases have been observed in which the smaller branch of a forking spike has every external appearance of being lateral in position, although it is evident that the stronger member of a pair of branches tends to straighten up and assume the appearance of a main axis. Several cases of branched spikes in *B. virginianum* have been observed in which the main stalk gives off a branch near its base, and at a point a half to one inch higher but on the opposite side of the stalk gives off a second branch. These triple spikes are most readily explained as cases of monopodial branching in which the two basal branches arise at a point lower than usual. Mr. Henry Mousley has very kindly sent me sketches of two specimens collected at Hatley, Quebec, each having three spikes, which appear to fall into this class. He has also been good enough to send a specimen in which one of the three branches of the spike undergoes a further division, giving the appearance of four branches of unequal sizes. It is a matter of common observation that in the various species of *Botrychium* the spike is broad and dense in some individuals, but skeleton-like or "stalky" in others, responding no doubt to differences in intensity of illumination. These stalky cases with long alternate branches merge imperceptibly into those which show what would at first sight be called cases of a double or triple spike. When the point of origin of two lateral branches is at about the same level, the true relation of the parts is still more disguised. *B. virginianum* is not the only species showing

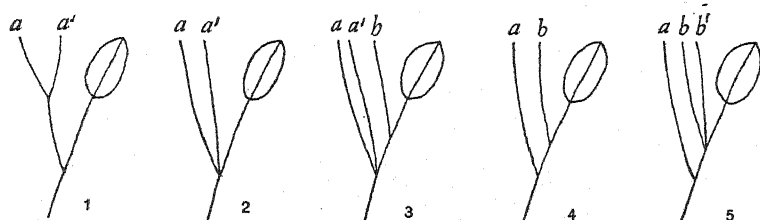
this remote alternate branching but *B. Lunaria* L., *B. dissectum* Spreng., *B. neglectum* Wood afford other cases. What might be called multiple fertile spikes represent the normal condition in *B. lanceolatum* Angstr. and *B. ramosum* (Roth) Ascherson, for in these species several branches of the spike arise near its base and close together although plainly alternate, giving the corymbed or bunched appearance characteristic of these species.

The possibility is recognized that cases exist where it is difficult to say whether remote monopodial branching or chorisis is the better explanation of the facts. But the cases which have come under observation seem to render it desirable to distinguish between the normal monopodial method of branching and the splitting which appears to be a result of an external agency.

The third group of cases which has been styled "reversion" has already been figured by the writer (8). Examination of all available material indicates that replacement of a single fertile spike by a pair of spikes is practically restricted to the *Ternatum* section of the genus, and has been observed in *B. obliquum* Muhl., *B. dissectum* Spreng., *B. silaifolium* Presl, and in other species which have been at times distinguished by students of this puzzling group. It is not surprising to find that this group which is in a taxonomic jumble should present features suggesting evolutionary phases. The 98 abnormal specimens which have been examined by the writer fall readily into the following groups: (1) the fertile spike forking equally part way up its stalk (FIG. 1); (2) a pair of fertile spikes, of equal development, arising right and left at the same level (FIG. 2); (3) a pair of spikes as in the preceding group, but with an additional spike arising somewhat further up (FIG. 3); (4) a normal spike, and a smaller additional one inserted somewhat further up (FIG. 4); (5) a normal spike, and a pair of smaller ones inserted somewhat further up (FIG. 5).<sup>1</sup> Of these forms, most of which have already been pictured by the writer (8), the fourth one occurs more frequently than any of the others, and it probably includes two classes, namely, cases where the upper (smaller) spike represents one pinna and where it represents two fused pinnae, as shown by the single or double vascular supply. That is, the strand supplying such spike may arise from one edge of the C-shaped leaf trace, or a strand may

<sup>1</sup> A photograph of a plant of *B. dissectum* representing this type has been published by Weatherby (17, pl. 1).

arise from each edge of the trace. So far, only plants of the first class have been sectioned. The reasons for regarding abnormal plants of *B. obliquum* as supporting the theory of Roeper (15) as to the morphological nature of the fertile spike, namely, that this represents a fused pair of pinnae, the basal ones of the leaf, have been stated in an earlier paper (8) and supported by additional evidence from *B. lanuginosum* (9). The recent examination of herbarium specimens from a wide range has served to confirm the writer in his opinion, and lead him to regard the cases here figured as belonging to a different category from the branching spikes of *B. virginianum et al.* Whether the double and triple spiked specimens of *B. obliquum* are to be regarded as reversions in a strict genetic sense may be open to question, but it is sug-



FIGS. 1-5. Diagrams to illustrate the methods of insertion of the fertile spikes in abnormal specimens of *Botrychium obliquum* Muhl. *a, a'*, branches of the normal spike, representing the first and second pinnae. *b, b'*, supernumerary spikes, representing the third pinna or third and fourth pinnae.

gested that leaves showing such abnormal spikes are reminiscent of the stages passed through by the ancestors of the Ophioglossaceae.

It would be of interest to determine whether plants showing extra spikes continue to show the peculiarity in subsequent years. It would not be difficult to settle this question if a person were located near a station where many abnormal specimens are available. Something of this kind was undertaken by Mrs. Scoullar (16) who marked two plants, and obtained the following results: "*Botrychium matricariefolium*: June 15, 1904 (fertile), June 18, 1905 (two fruiting spikes), June 16, 1906 (sterile), June 21, 1907 (fertile). *Botrychium obliquum dissectum*: Aug. 20, 1904 (fertile), Sept. 1, 1905 (fertile), Sept. 8, 1906 (sterile), Sept. 10, 1907 (two fruiting spikes)." These observations

plainly indicate that the production of "two fruiting spikes" is not hereditary. But another method of inquiry is open, on account of the well-known habit of *Botrychium* of laying down the primordia of several leaves in the bud enclosed in the base of the petiole. If the feature of producing extra spikes were to become fixed in the constitution of an individual, it should be visible in the bud, say in a plant like FIG. 4, for in *B. obliquum* the spikes arise so far down on the rachis that it should be possible to distinguish them from sterile leaflets even in the embryonic stage. Four plants from the writer's slender stock of abnormal plants have been studied from this point of view by means of serial sections through the bud. In each of these cases a single fertile spike appears to arise in an altogether normal manner. This negative evidence is recorded, in the absence of a satisfactory investigation of the matter. In view of Goebel's observation (10) of the permanency of an abnormality at a station on the Ostsee, one might expect that the double-spiked character would persist.

2. The occurrence of sporangia on pinnae ordinarily sterile is widely distributed among the species of *Botrychium*, and was observed by some of the early students of the group, e.g., Roeper (15) who in 1859 figured an example in *B. Lunaria*. As to the extent of this feature, the sporangia may be present in small numbers on the margin of one or more pinnae, or a whole pinna, usually a basal one, may be transformed into a fertile segment, or in extreme cases the whole lamina may be converted into a fertile organ. Goebel (10) mentions a locality on the Ostsee where this condition has become constant. It is obvious that the cases where a basal pinna is fertile approach those abnormal specimens of *B. obliquum* in which there is a supernumerary small fertile spike inserted between the ordinary spike and the sterile segments of the leaf. Goebel (*l.c.*) has used the occurrence of fertile pinnae to support the view that the regular fertile spike is also produced from part of a foliage leaf, and his argument has been followed by others, including the writer.

3. More or less complete sterilization of the fertile spike, like the second abnormal feature, was early observed and figured. Roeper (15) figures a plant of *B. Lunaria* in which the fertile spike is entirely transformed into a green leaf-like organ. Cases of partial sterilization occur in various species; specimens of *B.*

*cicutarium* (Sav.) Sw., *B. neglectum* Wood, *B. silaifolium* Presl, and *B. obliquum* Muhl. illustrating this peculiarity have recently come under the notice of the writer. Malformations of this third order show the necessity for caution in reading too much of the supposed history of a genus into these abnormalities, for it must be remembered that it is a poor rule that will not work both ways. Bower (6) has uttered a warning on this matter, reminding us that *all* malformations must be taken into consideration in framing a theory. Fortunately the interpretation of the fertile spike in *Botrychium* does not rest solely on the evidence from monstrosities, but depends on the disposition of the vascular structures and on other collateral evidence. Since Bower has added his support to the idea of relating Ophioglossaceae to the true ferns rather than to the Lycopsidea, we may consider that the consensus of opinion is in favor of regarding the fertile spike as an organ produced by the fusion of two basal pinnae of a fern leaf. The localization of spore-production in these basal pinnae implies derivation from ferns in which spore-production was not restricted to the basal part.

Localization of spore-production is in the majority of ferns apical rather than basal. A survey of common genera shows that when any part of the frond is sterile it is apt to be the basal region. Familiar examples are seen in the genus *Polystichum*. Whether the apical placement of sporangia is a light reaction ought to be open to determination by experiment. But it is easy to see how a relatively high location for sporangia would be of advantage in connection with dispersal of the spores, hence the teleological explanation readily occurs to one. Very few ferns have spore-production localized at the middle region of the leaf (e.g., *Osmunda Claytoniana* L.), and ferns in which the sporangia are situated on basal pinnae have these pinnae raised into a position favorable for dispersal of spores, e.g., *Anemia*, *Botrychium*.

It is convenient at this point to refer to certain peculiarities of the genus *Osmunda*. Although spore-production is fairly well localized in the apical region of the leaf in *O. regalis* and in the median region of *O. Claytoniana*, the oriental species *O. javanica* Blume may have its fertile part situated in the median, apical or even basal region, while some of the pinnae are intermediate between sterile and fertile, i.e., green pinnae show some sporangia

along one or both edges. This exceedingly variable species parallels *B. lanuginosum* (3, 7, 9), which has as a rule only one fertile pinna, occupying the position of second, third or higher pinna (up to the sixth at least), while in some specimens the fertile part is inserted at the base of the lamina<sup>2</sup> much as in *B. virginianum*. Inspection of a large series of specimens of *O. Claytoniana* shows that the number and location of fertile pinnae is subject to considerable variation; for instance, as few as two of the pinnae may be fertile, one or two of the fertile pinnae may stand opposite sterile pinnae, there may be no sterile pinnae below the group of fertile ones. The variation in extent of the terminal fertile region in *O. regalis* is of course familiar to all collectors. One specimen in the National Herbarium, collected in Miami, Florida, by Small and Nash, consists of a leaf which is entirely fertile, showing no green pinnules. Specimens exhibiting pinnules which are in part sterile and in part fertile are too common to merit more than passing mention. As for *O. cinnamomea* L., plants with leaves intermediate between sterile and fertile have sometimes been referred to as var. *frondosa* Gray. An inspection of forty-nine specimens of this "variety" shows six leaves sterile at the apex, thirty sterile at the base, ten sterile at both apex and base, and three transitional throughout. McLouth (13) reports the occurrence of "frondosa" stages in a swamp that had been burned over, to which cause he attributes the appearance of the intergrades. This explanation is rendered more probable by the experiment reported by Atkinson (1) who cut off the sterile leaves of *Onoclea sensibilis* L. as fast as they were produced (three times). In June and July these plants produced a large number of intermediate forms. Atkinson repeated the experiment with *O. Struthiopteris* Hoffmann, obtaining similar results. Dr. Kelley reports the finding of numerous intergrades in *O. sensibilis* in a pasture where the plants were browsed by cattle. The point of general import in these observations is the homology of fertile and sterile leaves and pinnae.

At least as far back as 1859 (Roepert, 15) the significance of the genus *Anemia* in interpreting the condition in *Botrychium* was perceived. The two basal pinnae functioning as fertile leaflets and extending vertically are striking and suggestive objects. Equally suggestive are some of the abnormal specimens which oc-

<sup>2</sup> Reported in a letter from Professor D. H. Campbell.



casionally occur. Reference has been made elsewhere (Chrysler, 9) to cases showing only one fertile pinna; some doubt might arise as to whether this condition might occur through accidental loss or through abortion of one of the spikes. But in certain specimens of *A. adiantifolia* (L.) Sw. having the pinnae plainly arranged in alternate fashion it becomes clear that only the first (basal) pinna is fertile. Closely related to these cases are others in which a fertile pinna is paired with a sterile one at the base of the leaf, as is seen in FIG. 7, from a photograph kindly furnished by Dr. M. A. Howe of the New York Botanical Garden, from a specimen collected by Shafer in Camaguey, Cuba. Other specimens of this species have three of the lowermost pinnae fertile (e.g., National Herbarium no. 755693) and another specimen on the same sheet as the preceding shows the normal pair of fertile pinnae and a third pinna with three of its pinnules fertile and the remainder sterile. Dr. W. H. Maxon has very kindly called my attention to two remarkable specimens in which there are six fertile pinnae (National Herbarium numbers 372323, 520050, one of which is represented in FIG. 6). An examination of the sheets at the New York Botanical Garden brought to light two specimens collected in Jamaica by Underwood, each of which has four fertile pinnae. Another highly interesting specimen (*Underwood 3300*) is represented in FIG. 8, from a photograph kindly furnished by Dr. M. A. Howe, and it will be seen that almost an entire leaf is fertile. The common species *A. adiantifolia* is evidently a very variable one, as is also *A. cuneata* Kunze. An additional point of interest in the specimen shown in FIG. 8 is the resemblance of this abnormal form to those species of *Anemia* in which heterophylly is established, e.g., *A. aurita* Sw. and *A. millefolia* Gardner; in fact one might be tempted on this basis to establish a probable evolutionary line within the genus.

These abnormal forms in *Osmunda*, *Onoclea* and *Anemia* plainly point to the necessity of interpreting at least some of the cases of multiple spikes in *Botrychium* as additional fertile pinnae.

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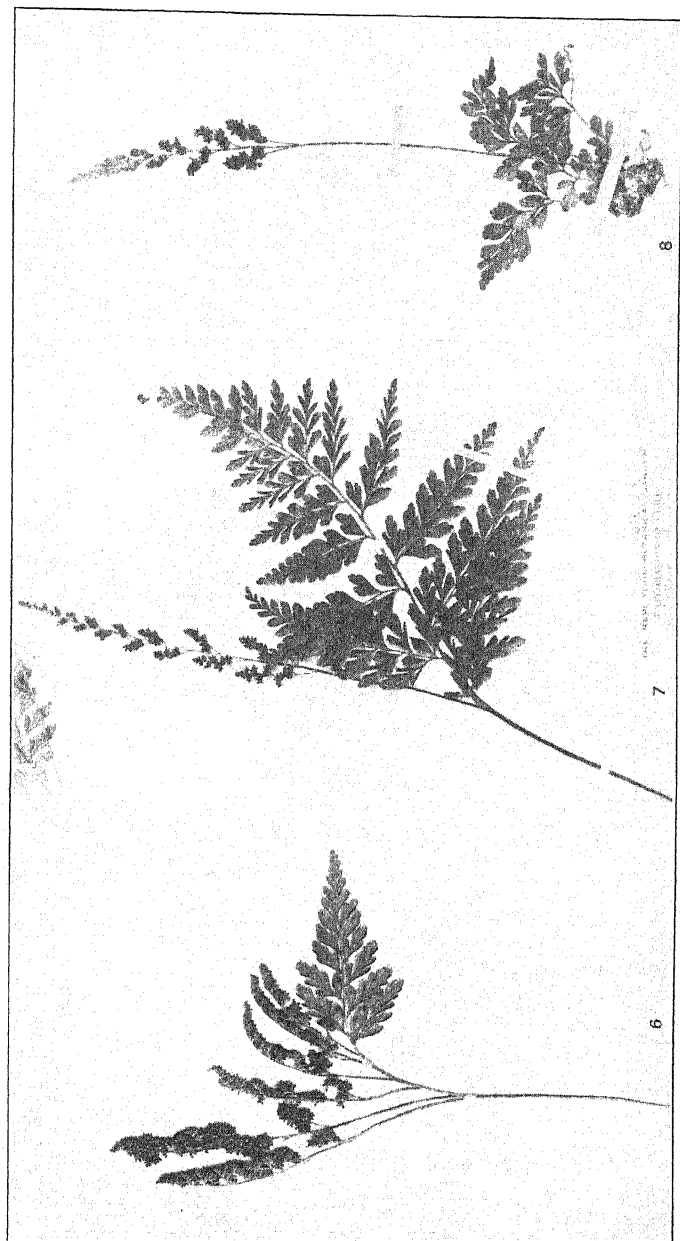
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## Explanation of plate 9

FIG. 6. *ANEMIA ADIANTIFOLIA* (L.) Sw.; specimen with four fertile pinnae in addition to the two normal ones (United States National Museum, no. 520050).

FIG. 7. *ANEMIA ADIANTIFOLIA* (L.) Sw.; specimen with one fertile pinna opposite a sterile pinna (New York Botanical Garden, *Shafer 1152*).

FIG. 8. *ANEMIA ADIANTIFOLIA* (L.) Sw.; specimen with one leaf almost wholly fertile (New York Botanical Garden, *Underwood 3300*).



ANEMIA ADIANTIFOLIA (L.) SWARTZ



## Studies on the flora of northern South America—IX<sup>1</sup>

H. A. GLEASON

### MISCELLANEOUS NEW SPECIES FROM BRITISH GUIANA

**Tetrapodenia** n. gen. Malpighiaceae; arborescent, the young branches strongly flattened; stipules axillary, connate, erect; leaves opposite, petiolate, coriaceous, entire; flowers in minutely bracted, terminal racemes, on jointed pedicels; bracteoles 2, the lower glandular; calyx 10-glandular; petals 5, clawed, four essentially alike, the fifth much smaller, its blade bearing two pairs of latero-basal, stipitate, reniform glands; stamens 10, the five opposite the sepals the longer; filaments short, glabrous, somewhat triangular-dilated and barely connate at base; anthers elongate, the stout, simple, semicylindric or triangular-prismatic connectives truncate to broadly rounded at the apex; pollen-sacs 4, elongate, strictly parallel in the longer stamens, slightly divergent distally in two pairs in the shorter; ovary depressed-hemispheric, 3-celled, glabrous; styles 3, glabrous, bent near the tip at right angles and gradually tapering distally to the obliquely truncate terminal stigma; fruit indehiscent, 1-celled and 1-seeded by abortion, subellipsoid, the exocarp thick and woody, the endocarp chartaceous; seed ellipsoid, the small cotyledon partly enclosed by the large.

The name is combined from τέτρα, πούς, and ἄδην, referring to the four stalked glands on the fifth petal.

**Tetrapodenia glandifera** n. sp. Tree 4-6 m. high; young branches strongly compressed, glabrous, later becoming terete and roughened by elongate lenticels; stipules triangular-subulate, erect, stiff, 6-8 mm. long, densely pubescent above; petioles 10-15 mm. long, glabrous, flattened or barely channeled above; leaf-blades stiff, coriaceous, dull green, elliptic-oblong, 10-17 cm. long, 4.5-8.5 cm. wide, abruptly acute or subacuminate, entire, broadly cuneate to obtuse or rounded at base, glabrous above, glabrous beneath (except under strong lens minutely lepidote) and biglandular at the base; veins and veinlets prominent on both sides, conspicuously reticulate; racemes single or in threes, terminating the branches, the axis somewhat flattened and minutely puberulent, the bracts broadly ovate-triangular, 1-2 mm. long; pedicels 7-8 mm. long, glabrous, jointed in the middle, bearing on the lower segment two broadly ovate, amplexicaul bracteoles 1.5 mm. long, the lower of which bears a conspicuous gland; calyx-glands 10, cuneate-obovate, adnate to

<sup>1</sup> Contributions from the New York Botanical Garden, no. 282.

the summit of the pedicel; sepals triangular, acute or barely retuse, exceeding the glands by 1.3 mm.; petals dimorphic, clawed, the four larger with semiorbicular, subentire blade, cleft at base to the insertion of the claw, the fifth much smaller, obovate-oblong, with stout fleshy claw, the basal pair of glands 0.6 mm. in diameter, on stipes 0.5 mm. long, the distal pair half as large, on stipes one third as long; filaments flat, glabrous, 3 or 2 mm. long, dilated and barely connate at base; anthers stout 2-2.5 mm. long in bud, the simple connective somewhat surpassing the narrowly linear pollen-sacs; fruit smooth, 20-25 mm. long by 12-15 mm. in diameter, rounded at base, subacuminate to the blunt apex which is marked by 6 faint radial furrows.

Type, *La Cruz 3515*, collected at Amakura, Northwest District, British Guiana, 23-30 March 1923, and deposited in the herbarium of the New York Botanical Garden. *La Cruz 2176*, from the upper Mazaruni River, collected the preceding autumn, agrees with the type in all vegetative characters, except some small differences in texture due to age, and exhibits mature fruit and old flowers from which the petals have fallen. *La Cruz 2869*, with mature fruit, and *2043*, sterile, both from the Mazaruni region, differ from the first two in their larger, proportionately narrower, and more sharply acute leaves which are distinctly shining above. The fruit is precisely the same. The specific description is drawn entirely from the type, except that the length of the filaments is measured from the withered flowers of *2176*. Duplicates of all four numbers are to be found in the leading American herbaria.

*Tetrapodenia* is obviously closely related to *Glandonia* and *Burdachia*, two genera of the northern Amazonian forests which are still poorly known and have probably not been collected in recent years. The former is monotypic, but both flowers and fruit are known and were carefully described by Grisebach in the *Flora Brasiliensis*. The latter contains two species, but the flowers of only one are known. *Tetrapodenia* differs from *Glandonia* in its hirsute stipules, the glands on the lower surface of the leaf, the differentiated fifth petal, the unappendaged anther-sacs, the shape of the fruit, and the presence of two cotyledons. From *Burdachia prismatocarpa*, it is distinguished by the glabrous, depressed-hemispheric ovary, the simple blunt connectives, the character of the fifth petal, and the shape of the fruit. From *Burdachia sphaerocarpa*, the flowers of which are not known, it differs in its glabrous stipules and the shape of the fruit.

*Tetrapodenia glandifera* is represented in the herbarium of the Royal Botanic Garden at Kew by two collections by Jenman, 3779 and 6413.

**Combretum brunnescens** n. sp. Shrubby vine, climbing 6 m.; stems glabrous, strongly furrowed and flattened above, becoming terete and barely striate in age, the upper internodes 5 cm. long; petioles stout, dark brown, 6–10 mm. long, angular, glabrous; leaf-blades coriaceous, olive-brown when dried, broadly elliptic-obovate, 70–85 mm. long, 43–58 mm. wide, obtuse to subrotund at base, entire, broadly rounded above and minutely apiculate, shining above, glabrous on both sides; lateral veins 5 or 6 pairs, lightly impressed above, prominent beneath, ascending at an angle of 60° and nearly straight, the veinlets obscure; panicles freely branched, terminal and from the upper axils, 10 cm. long; spikes spreading, 5–6 cm. long, the rachis strongly angled, glabrous; bracts subulate, puberulent, 0.5–1.1 mm. long; ovary sessile, linear-oblong, 1.1–1.3 mm. long, 0.4 mm. in diameter, obtusely 4-angled, glabrous or minutely villous; calyx campanulate, glabrous, conspicuously but minutely red-dotted, 1.7 mm. long, including the broadly triangular, acute teeth 0.4–0.5 mm. long, glabrous within; petals white, transversely elliptic, 1.4 mm. wide, 0.8 mm. long, glabrous, with a short claw 0.1 mm. long; stamens 8, inserted near the bottom of the calyx, the filaments white, glabrous, subulate, 4 mm. long, somewhat incurved at three-fourths of their length, the anthers obcordate, 0.4 mm. long and wide; disk a minute glabrous ring at the bottom of the calyx; style straight, white, subulate, 2.8 mm. long.

Type, *La Cruz* 3566, collected along the Amakura River, Northwest District, British Guiana, lat. about 8° 10' N., long. about 60° W., 23–30 March 1923, and deposited in the herbarium of the New York Botanical Garden.

The type of *Combretum brunnescens* has been chosen arbitrarily from several specimens in the herbarium of the New York Botanical Garden, all collected in the same general region. Duplicates of most of them have been distributed to the larger American herbaria. These are *La Cruz* 1094 and 1141 from the Pomeroon District, 3425, 3585, and 3713 from the Northwest District, 1947 from near Bartica, 4258 from Kamakusa, upper Mazaruni River, and 4460 from Kaieteur Falls, Potaro River. All but the last two of these are in flower and differ from the type only in minor details. In two from the Pomeroon District the largest leaves are only 6 cm. long; in all except 1947 and 3425 the leaves are more shining above; in 1141, 1947, 3585, and 3713 the terminal apiculum of the leaf is more developed and

as much as 5 mm. long; in 1947 the largest leaves are 73 by 100 mm.; in 3425 the rhachis is sparsely villous. I have considered these discrepancies as representing merely the variability of the species and not worthy of taxonomic differentiation. Another sheet, *La Cruz 4460*, from Kaieteur Falls bears malformed fruits, and the leaves reach 103 by 140 mm. in size. *La Cruz 4258* is the only fruiting specimen: the leaves measure as much as 75 by 100 mm. and the fruit is ovoid, 18 mm. long by 11 mm. in diagonal diameter, strongly 4-winged, on a stipe 5 mm. long, and narrowed above to a subacute apex.

Through the key in *Flora Brasiliensis*, our species appears related to *C. Jacquinii* Griseb., a West Indian species properly known as *C. laxum* Jacq. It differs in the leaf-shape, in the fruit, and in the calyx glabrous within.

***Combretum fusiforme* n. sp.** Stems terete, glabrous, the internodes 7-8 cm. long; petioles slender, glabrous, angular, 5 mm. long; leaf-blades thin but firm, olive-green, elliptic-oblong, 8-11 cm. long, 32-42 mm. wide, broadest slightly above the middle, rounded at the base, conspicuously acuminate, glabrous, somewhat shining above, dull green and minutely punctulate beneath, lateral veins about 7 pairs, arising at an angle of 80° and curved-ascending, veins and veinlets elevated on both surfaces and prominently reticulate; inflorescence and flowers lacking; fruit brown, fusiform, 40-48 mm. long, 8-11 mm. wide, acuminate to both ends, almost square in cross-section, sharply 4-angled, somewhat depressed along the center of each side, the cavity almost circular in cross-section.

Type, *La Cruz 4104*, collected at Kamakusa, upper Mazaruni River, British Guiana, long. about 59° 50' W., 11-22 July 1923, and deposited in the herbarium of the New York Botanical Garden.

*Combretum Sprucei* Eichl. is the only other species known to me with fruit of similar size and proportions. It differs from *C. fusiforme* in having a sharply winged fruit with the sides projecting into the cavity.

***Combretum fulgens* n. sp.** Stem shrubby, climbing, somewhat striate above and flattened at the nodes, terete when older, glabrous; petioles stout, thinly pubescent with simple brown hairs, 6-7 mm. long; leaf-blades firm or subcoriaceous, olive-green when dry, shining above, dull beneath, oblong to ovate-lanceolate, 11-12 cm. long, 40-52 mm. wide, broadest at or slightly below the middle, obtuse or subrotund at base, short-acuminate, glabrous on both sides, lateral veins 10-12 pairs,



lightly impressed above, elevated beneath, arising at an angle of 70° and gently curved-ascending, continuous almost to the margin, veinlets obscure above, faintly reticulate beneath; inflorescence of simple spikes in the upper axils, 5-8 cm. long, and sparingly branched terminal panicles 12 cm. long, the rhachis thinly pubescent with simple hairs; flowers not seen; fruit turbinate, 6-7 mm. long, 10-11 mm. in diagonal diameter, sharply 4-winged, rounded at base, truncate above, the lateral faces bearing 1 or 2 low irregular ridges in the distal half.

Type, *La Cruz 4129*, collected at Kamakusa, upper Mazaruni River, British Guiana, long. about 59° 50' W., 11-22 July 1923, and deposited in the herbarium of the New York Botanical Garden. Specimens were distributed to various American herbaria under the name *Combretum nitidum* Spruce, to which it is closely related. In that species, as represented by *Spruce 1482* at Kew, the fruit is only half as large, not truncate at the apex, and without the conspicuous lateral ridges, the leaves are less shining, with lateral veins twice as far apart, and more acuminate, and the petioles are densely gray-lepidote.

**Cybianthus Brownii** n. sp. Shrubby, 3 m. tall; branches slender, irregularly angled, densely red-lepidote at the apex, sparsely so a few centimeters from the tip, and eventually glabrous; petioles slender, about 15 mm. long, finely and rather densely lepidote; leaf-blades bright green, thin, narrowly obovate to almost oblanceolate, abruptly subacuminate to an acute tip, entire, cuneate from above the middle to an acute base, sparsely black-punctate above, the lower surface more or less thickly red-dotted, closely beset with minute white glands, and sparsely black-punctate; veins elevated on both surfaces, finely reticulate; inflorescence racemose or subspicate, axillary, the staminate racemes 15-20 cm. long, rhachis sharply and irregularly angled, minutely and closely glandular-puberulent; bracts deciduous, subulate, 1.5-2 mm. long; pedicels stout, 1 mm. long; sepals firm, ovate, 1.5 mm. long, connate for one-third or more of their length, subacute, glabrous, conspicuously black-punctate; petals spreading, connate for more than half their length into a quadrangular or nearly square corolla, 4 mm. in diameter, the lobes broadly depressed-deltoid-ovate, obtuse or rounded, conspicuously punctate, especially at the margin; anthers inserted at one-third the length of the petals, strictly sessile, broadly flattened-ovoid, 0.6 mm. long and wide, with a large black gland on the back, dehiscing by two subintrorse terminal elliptical pores.

Type, *Gleason 159*, collected in dense upland forest, Tumatumari, British Guiana, 18 June to 8 July 1921, and deposited in the herbarium of the New York Botanical Garden. The

leaves on the type vary from 20 to 30 cm. long by 72 to 105 mm. wide. Here are referred also *Gleason* 723, from Butukari, on the Essequibo River (staminate); *Jenman* 3977, from the upper Demerara River (staminate); *La Cruz* 2665, from Malali, on the Demerara River (staminate); and *La Cruz* 1601 and 1648, from between the Demerara and the Berbice Rivers, near Wismar (fruiting). In the latter four sheets the leaf-blades are somewhat smaller than in the type, averaging 15–20 cm. long. The fruiting racemes are shorter than the staminate, mostly 4–6 cm. long; the mature berry is globular, 6 mm. in diameter, and densely verrucose-glandular.

Mez, in preparing his monograph, apparently did not examine *Jenman* 3977, the only specimen known to me which antedates his work. Our species, with its strongly punctate petals connate to well beyond the middle, is related, according to Mez' treatment, to *C. venezuelanus* Mez, but differs in its shorter pedicels, its remarkably broad petals, and in other details.

The species is named in honor of Mr. N. E. Brown, long a curator of the herbarium of the Royal Botanic Gardens at Kew, who has been of the greatest assistance in our studies of the plants of British Guiana.

***Weigeltia sylvatica* n. sp.** Shrubby, 4 m. high; floriferous branches stout, irregularly angled, black-punctate and minutely fulvous-lepidote at the apex, glabrous below; petioles stout, glabrous, minutely rugulose, 12–15 mm. long; leaf-blades stiff, firm or coriaceous, reddish-brown when dried, elliptic-oblong to subobovate-oblong, 20 cm. long, 73–93 mm. wide, abruptly subacuminate to an acute tip, entire, acute and conspicuously inequilateral at base, glabrous on both sides, freely black-punctate along the midrib above, the lower surface very sparsely black-punctate, freely but minutely fulvous-lepidote, and densely and minutely white-punctate, the veins elevated and conspicuously reticulate on both sides; inflorescence axillary, virgately paniculate, the axis 4–11 cm. long, sharply angled, minutely puberulent with stout subcapitate hairs, the branches 3–15 mm. long, with 2–13 crowded flowers; bractlets subulate, 2 mm. long; pedicels stout, glabrous, 1 mm. long; flowers 4-merous; calyx 1.6 mm. long, the sepals connate for one-fifth of their length, the lobes oblong-triangular, 1.3 mm. long by 0.4–0.7 mm. wide, acute, entire, not punctate; petals 1.9 mm. long, connate for one-third of their length, the free portion ovate, 1.2 mm. wide, acute, entire, sparsely punctate; filaments inserted 0.5 mm. above the base of the corolla, truncate-triangular, 0.3 mm. long

and almost as broad; anthers dorsifix near the middle, depressed-ovate, 0.5 mm. long, 0.6 mm. wide, obtuse or truncate, not punctate, dehiscent by two longitudinal slits; ovary of the staminate flowers ovoid, glabrous.

Type, *Gleason 271*, collected in dense upland forest between Kangaruma and Potaro Landing, on the Potaro River above Tumatumari, British Guiana, 25-27 June 1921, and deposited in the herbarium of the New York Botanical Garden. According to the arrangement of species in Mez' monograph, *W. sylvatica* takes a position near *W. Schomburgkiana* Mez, from which it differs in the shape and texture of its leaves, the length of the pedicel, the pubescence of the rhachis, and the shape, size, margin, and punctuation of the sepals and petals.

Bentham in 1876 described a new genus *Lissocarpa* of the family Styracaceae, based on Spruce's two collections, 3108 and 3504, from the upper Rio Negro in northern Brazil. It is quite probable that these two specimens remained for a long time the only ones extant under that name, although the Kew herbarium contains two other old collections, one by Parker and the other *Jenman 4955*. The description was prepared with the usual detail of the Genera Plantarum, but may not have been entirely accurate, from lack of sufficient material. Gürke used Bentham's description of the genus in his presentation of the family in *Die Natürlichen Pflanzenfamilien*, although he probably did not see actual specimens, and supplied the plant with a specific name *Benthami*. Oliver published a plate and a page of description in 1895 (Hooker, *Icones Plantarum*, *pl. 2413*), in which he regrets that because of lack of material he was unable to ascertain satisfactorily the structure of the androecium. Miss Perkins, in her monograph of the family Styracaceae for *Das Pflanzenreich*, excluded *Lissocarpa*, but confessed that she was unable to suggest another place for it. Oliver, who admitted the genus to the Styracaceae, indicated its general resemblance to the Ebenaceae, apart from its wholly inferior ovary, a character found likewise in *Halesia* of the Styracaceae. The present writer believes the genus should remain where it was originally placed by Bentham.

Abundant material of the genus was collected by the writer in 1921, under the number 724, from a single plant, a small tree perhaps twenty feet high. It was in full bloom, that is, the twigs were crowded with unopened flower-buds and the ground

beneath was carpeted with fallen corollas. No open corollas were observed on the tree. They are pure white, not "sordide lutei" as stated by Benthham and repeated by Oliver from their examination of dried material. Since then three other collections have been received from British Guiana.

Mr. N. E. Brown, on examining our collection, wrote: "A very distinct new species, with much larger flowers and leaves than *L. Benthami* has." Nevertheless, it was placed in our herbarium under Gürke's name, and it was not until the writer examined Spruce's plants personally, at Kew in 1924, that its specific distinctness was admitted. The two species may be distinguished as follows:

Leaves barely acuminate to a decidedly obtuse tip; midvein scarcely prominent above; lower leaf-surface distinctly dull-green or glaucous-brown, the veinlets obscure; exposed portion of corolla in mature buds 7 mm. long. *L. Benthami*

Leaves strongly acuminate to a sharply acute tip; midvein equally prominent on both sides; lower leaf-surface the same color as the upper, with very prominent, conspicuously reticulate veinlets; exposed portion of the corolla in mature buds 14 mm. long. *L. guianensis*

***Lissocarpa guianensis* n. sp.** A small tree 6 m. or more high; leafy twigs finely roughened and mottled light and dark gray; petioles 5-7 mm. long; leaf-blades firm, dull-green, drying bronze-green, narrowly elliptic-oblong, 17-22(14-23) cm. long, 52-68 (41-92) mm. wide, strongly acuminate to a sharply acute tip, entire, cuneate to an acute (or occasionally subrotund) base, glabrous and shining with prominently elevated, strongly reticulate veins and veinlets on both sides; flowers in short, dense cymes in the axils or a few supra-axillary; pedicels 2-5 mm. long; bractlets 2, closely subtending the hypanthium, broadly rotund, somewhat scarious at the margin; flowers 5-merous; calyx and hypanthium narrowly obconic, 10-11 mm. long, the ovary wholly inferior, 4 mm. long, the calyx-tube thick and fleshy, 4 mm. long, the calyx-lobes broadly rotund, 2 mm. long, erect, rounded or somewhat retuse, minutely erose-denticulate; corolla salverform, pure white, drying almost black, fleshy, the tube obconic, 10 mm. long, the lobes elliptic, 16 mm. long by 8 mm. wide, obtuse, convolute in bud; stamens 8, inserted on the corolla-tube 3 mm. from its base, erect, included; filaments 1 mm. long; anthers linear, 4.7 mm. long, acuminate to a blunt tip, 2-celled, longitudinally dehiscent; coronal tube inserted on the corolla 6 mm. from its base, entire to the throat, distally cleft into 8, elliptical-oblong, sharply acute, projecting lobes 6 mm. long by 2 mm. wide; style slender below, thickened upward to the capitate stigma.

Type, *Gleason 724*, collected at Butukari, on the Essequibo

River, British Guiana, 20-21 July 1921, and deposited in the herbarium of the New York Botanical Garden. Other collections in the same herbarium are *Persaud 154*, from Hyde Park, near Georgetown, *La Cruz 3076*, from the upper Mazaruni River, and *La Cruz 3356*, from the Barima River, Northwest District. The dimensions stated in the description are taken from the type, the maximum and minimum sizes of the leaves are from the other three specimens.

It is difficult to contrast the flower-structure of the two species by reference to Oliver's plate, since he speaks of the scarcity of material for dissection, but the corolla-lobes of *L. guianensis* seem much longer in proportion to the tube, and also narrower and sharper than in *L. Benthami*, while the lobes of the coronal tube are narrower, sharper, and proportionately much longer. Our dissections answer one doubtful point raised by Oliver: the stamens are quite free from the coronal tube and inserted on the corolla-tube some distance below it.

Among the numerous interesting plants collected in British Guiana by J. S. De La Cruz are two sheets of the same species, illustrating a plant with such remarkable floral structure as to demand the erection of a new genus.

**Barnhartia** n. gen. Shrub or small tree; leaves alternate, petioled, simple, entire; flowers in simple or paniced raceme-like spikes, perigynous, somewhat zygomorphic; disk none; sepals 5, strongly imbricate, inserted on the summit of the hypanthium, distinct, pubescent; petals 5, valvate in the bud, clawed, inserted on the summit of the hypanthium, strongly hirsute within, 4 somewhat connate at base into two pairs, the fifth free and distinct; stamens 7 or 8, epipetalous, 5 opposite the petals and 2 or 3 alternate with them, each pair of petals bearing 2 opposite stamens and 1 alternate one, the odd petal bearing 1 opposite and sometimes 1 lateral alternate stamen; filaments short, flat, inserted near the summit of the claw; anthers flattened-ellipsoid, 2-celled, opening by a tangential cleft; ovary wholly superior, attached by a broad base, slightly flattened, 2-celled; ovule single in each cell, pendulous; style terminal, straight, pilose; stigma capitate, 2-lobed.

**Barnhartia floribunda**, n. sp. Stems minutely puberulent above, soon becoming glabrous, the internodes 1-2 cm. long; petioles nearly black, 5-10 mm. long, wrinkled or verrucose, glabrous; leaf-blades firm or subcoriaceous, bright green, narrowly elliptic-oblong, 10-12 cm. long, 3.5-4 cm. wide, subacuminate to an acute tip, entire, cuneate at base, glabrous, the veins and veinlets prominently elevated and conspicuously

reticulate on both sides; inflorescence of 1-3 simple or paniced raceme-like spikes from each of the upper axils, 2-5 cm. long, forming a terminal leafy panicle 10-15 cm. long, the axes thinly but densely gray-pubescent; bractlets subulate, 1.5 mm. long; flowers sessile, or on pedicels 1 mm. long; bracteoles broadly round-ovate, blunt, pubescent like the axis, 0.5 mm. long, closely subtending the hypanthium; hypanthium obconic, closely pubescent, nearly 1 mm. long; sepals thick, elliptic-oblong, 3.5-4 mm. long, densely pubescent or subtomentose; petals somewhat fleshy, linear-spatulate, 6.5 mm. long, the claw 3-3.2 mm. long by 0.5 mm. wide, orange-color, densely hirsute within, the blade maroon, 1.2 mm. wide, broadly rounded at the summit, hirsute within, the two pairs connate for half the length of the claw; filaments 0.5 mm. long, concealed in the pubescence of the claw; anthers 0.6 mm. long, the introrse valve soon shriveled and pendent; ovary 1 mm. in diameter, faintly 2-lobed; style straight, stout, 4-4.5 mm. long, pilose; stigma 0.7 mm. in diameter; fruit unknown.

Type, *La Cruz 2852*, collected at Kamakusa, upper Mazaruni River, British Guiana, 23-29 Nov. 1922, and deposited in the herbarium of the New York Botanical Garden. The type is in full bloom; a second sheet, *La Cruz 2727*, from Malali, on the Demerara River, was collected about a month earlier and is in bud. The two numbers differ only in unessential features, the leaves of the second reaching 13.5 cm. in length. Duplicates of both numbers are located in the principal American herbaria.

The arrangement of the petals in two loosely and partially connate pairs with a fifth one free is remarkable and is apparently the cause of the reduction in the number of the stamens from ten to seven or eight. All five petals are free and distinct at the level of the insertion of the short filaments. Each pair of petals bears three stamens, one at the center of each petal and a third on the margin of one of them toward the other member of the pair. There is no regularity in the location of the bistaminate petal. It may be either the posterior or the anterior of the two. The fifth or odd petal bears either a single central stamen, making a total of seven, or also a second lateral stamen, making eight for the whole flower. The three sinuses between the petals are somewhat wider than the width of the claw, and are obvious even in the unopened buds after dissection. The union of the paired petals is very weak and easily broken during dissection.

It is at once apparent that the three missing stamens, in the flowers with seven, are those which stand opposite the three

sinuses of the corolla. The two stamens of this set which are regularly present stand between the members of the pairs, but are always attached to the inner face of one petal at its margin. The eighth stamen, when present, has a similar marginal position on the fifth or odd petal. The slight connation of the paired petals is facilitated by their approximation on the margin of the hypanthium away from a normal position alternate with the sepals, and each of the pairs is exactly subtended by a sepal. The superior sepal subtends the sinus between the pairs of petals, while the fourth and fifth, or anterior, sepals subtend the sinuses between the pairs and the fifth petal and overlap behind it.

The complete epipetaly of the stamens and the tendency toward gamopetaly, or rather the tendency away from gamopetaly toward choripetaly, suggest at once the relationship of the genus to the *Styracaceae*. In that family, linear stamens and complete gamopetaly are the rule, but in the anomalous genus *Diclidanthera* the petals are only weakly gamopetalous and the anthers are precisely similar to those of *Barnhartia*. *Diclidanthera*, on the other hand, has a full complement of ten stamens, a salverform corolla, and a 5-celled ovary. Miss Perkins, in her monograph of *Styracaceae* for Das Pflanzenreich, rejected *Diclidanthera*, without suggesting a better position for it, and *Barnhartia*, which is certainly related, is additional evidence of their anomalous position.

The genus is dedicated to Dr. John Hendley Barnhart, whose extensive knowledge of botanical bibliography and biography has enabled him to render important service to his colleagues and his science.

***Prestonia guianensis* n. sp.** Stems woody, twining, minutely verruculose, otherwise glabrous, the internodes 5-10 cm. long; stipules about 6 at each node, sharply triangular, 0.6 mm. long, subscarios; petioles 5-7 mm. long, glabrous or verrucose, channeled above; leaf-blades firm or subcoriaceous, ovate-oblong, 8-10 cm. long, 4-5 cm. wide, or the uppermost somewhat smaller; sharply and abruptly acuminate, entire, subrevolute, rounded at base, upper surface dark dull green, glabrous, lower surface brownish green, minutely scabrously roughened on the veins and veinlets; venation lightly reticulate above, strongly reticulate beneath, midvein and primary veins shallowly impressed above, the veinlets plane, all very prominently elevated beneath; primary veins about 8 pairs, abruptly and arcuately

anastomosing near the margin; peduncles axillary, 3-4 cm. long, glabrous; pedicels (and rhachis) minutely puberulent, 10-15 mm. long, in a crowded raceme, subtended by 2 or 3 minute, subulate, pale bracts; sepals ascending, elliptic-ovate, 9 mm. long, 4 mm. wide, acute, glabrous, subprominently longitudinally veined, each with an interior, basal, broadly triangular, subacute, glabrous scale almost equaling the disk; corolla-tube cylindric, stout, 13 mm. long, glabrous without, softly villous within on the upper 4 mm., conspicuously thickened into a cartilaginous ring at the throat, unappendaged; corolla-limb spreading, the lobes 12 mm. long, broadly rounded at apex, glabrous, a basal central ovate-elliptic portion 9 mm. long, acute, reticulately veined, and comparatively firm, the distal portion very thinly membranous; filaments 3 mm. long, the basal portion filiform and densely villous, the distal portion nearly glabrous and obtriangularly flattened; anthers linear-subulate, cohering in a cone, 3.5 mm. long over all, protruding 2 mm. from the corolla-tube, polliniferous in the distal half, minutely pubescent dorsally on the central half, united to the stigma by a narrow introrse ventral ring at the center, prolonged below at the sides into linear-subulate caudae 0.7 mm. long and at the middle into a triangular-acuminate, scarious membrane of the same length; style minutely subclavate at apex, slightly thickened toward the base; disk fleshy, 1.5 mm. long, 5-lobed to the base, the lobes equaling the ovary, glabrous, ovoid, truncate and minutely 2-4-crenulate at apex; ovaries ovoid, subacute, glabrous; fruit not seen.

Type, *La Cruz 3097*, collected on the Pomeroon River, Pomeroon District, British Guiana, and deposited in the herbarium of the New York Botanical Garden. Duplicates are located in the Gray Herbarium and the United States National Herbarium. It was noted by the collector as climbing four feet high; the corolla is apparently yellow. There can be no doubt of its position in the genus *Prestonia*, although the small scale-like appendages at the throat of the corolla are missing. I know of no other species in the genus to which it bears a close similarity.

***Odontonema macrophyllum* n. sp.** Stems shrubby, 1-2 m. tall, leafless below, obscurely angled above, glabrous or nearly so; leaf-blades bright green, thin and membranous, obovate-elliptic, the principal ones 20-23 cm. long by 7-9 cm. wide, sharply acuminate into a slender tip, entire, cuneate from near the middle to a narrowly alate, pubescent petiole 2-4 cm. long, minutely and sparsely puberulent on the upper surface with appressed evanescent hairs 0.1-0.2 mm. long (and in no. 213 also with scattered, flat, crooked hairs 1.5-2 mm. long), pubescent below



with flat, crooked hairs 1.5–2 mm. long scattered over the surface and crowded along the principal veins; racemes lax, 25–30 cm. long, floriferous on the upper half, the axis, bracts, pedicels, and calyx closely pubescent with spreading, purplish, conspicuously septate hairs 0.5 mm. long; bracts linear-subulate, 5 mm. long; pedicels single and opposite or in opposite pairs, gradually elongating at anthesis to 7 mm.; calyx-tube 1.2 mm. long; sepals linear-triangular, 8 mm. long; corolla red, the tube minutely puberulent, 4 cm. long, the lobes 5 mm. long, obtuse, ovate.

Type, *Gleason 213*, collected in an Indian clearing at Kanagaruma, on the Potaro River above Tumatumari, British Guiana, 25–27 June 1921, and deposited in the herbarium of the New York Botanical Garden. A second sheet, *Gleason 7*, from wet lowland forest at Tumatumari, 18–20 June 1921, lacks the longer hairs on the upper leaf-surface.

The species is closely related to *Odontonema Schomburgkianum* (Nees) Kuntze (*Thyrsacanthus Schomburgkianus* Nees), the original material of which has been examined in the Kew Herbarium. There the leaves are distinctly smaller, thicker, narrower, and more gradually cuneate to the base, the larger ones averaging 12–17 cm. long by 3.5–4.5 cm. wide, the stem is conspicuously angled, the raceme less pubescent, and the narrower sepals are only 4–5 mm. long. Recent collections of Nees' species from British Guiana are *Gleason 699*, *La Cruz 2989*, and *La Cruz 137*.

### Additions to the genus *Lycianthes*—A correction

In the title of my article on the genus *Lycianthes* in the April number of the BULLETIN (53: 209-213) I omitted placing the name Dunal in parentheses, followed by that of Hassler, who elevated *Lycianthes* to generic rank. I should also have said that most of the later described species, rather than the whole of *Brachistus*, were transferred to *Lycianthes* by Bitter.

I find that the name *Lycianthes ferruginea* is preoccupied. My *Bassovia ferruginea* will therefore require renaming, and I substitute the name *Lycianthes rufinervia*.

H. H. RUSBY.

# Contributions to the flora of Long Island, New York

## Fourth paper<sup>1</sup>

WILLIAM C. FERGUSON

The plants listed below represent species collected during 1925 that, in the writer's experience, are from very rare to uncommon. In addition, localities are given for species not uncommon, where the records furnished by Mr. Norman Taylor, of the Brooklyn Botanic Garden, show one, two, or possibly three localities. All plants except three or four of the more common ones were collected by the writer from all the localities named, and specimens from each locality are in his herbarium. With one or two exceptions duplicates of all the rarer plants have been deposited in public herbaria. This is also true of the rarer plants listed in former papers. All critical specimens have been reviewed by professional botanists specializing in such plants.

The writer acknowledges with grateful appreciation this generous aid which adds so much to the permanent value of what is recorded.

Mrs. Agnes Chase has reviewed all the grasses.

Mr. Kenneth K. Mackenzie has reviewed *Carex*.

Professor T. G. Yuncker has reviewed *Cuscuta*.

Professor M. L. Fernald, and other authorities, whose names appear with the plants determined or corroborated by them, have reviewed many specimens.

### POLYPODIACEAE

*DRYOPTERIS HEXAGONOPTERA* (Michx.) C.Ch. Hilly, rich woods; uncommon. Millneck; North Deer Park; Port Washington; Queens; Plattsdale; Bayside; Southold.

### LYCOPODIACEAE

*LYCOPodium LUCIDULUM* Michx. Wet and damp rich and mostly hilly woods; rare. Plattsdale; Wyandanch; Farmingdale; Smithtown; Millneck; Cold Spring Harbor.

### SPARGANIACEAE

*SPARGANIUM EURYCARPUM* Engelm. Swamps; rare. Montauk (two or more stations); Winfield.

<sup>1</sup> Previous papers in this series appeared in *Torrey* **22**: 43-49. 1922; *Bull. Torrey Club* **51**: 177-201. 1924; *Bull. Torrey Club* **52**: 133-136. 1925.

## VALLISNERIACEAE

*PHILOTRIA NUTTALLII* (Planch.) Rydb. Determined by P. A. Rydberg. Streams and ponds; uncommon. Cold Spring Harbor; Millneck; Woodside; Mattituck.

## GRAMINEAE

*SPARTINA PATENS CAESPITOSA* (A. A. Eaton) Hitch. Massapequa: salt marsh. "Larger than type; not caespitose, shows short rhizome"—Mrs. Chase.

*STIPA AVENACEA* L. Dry oak woods; rare. Hither woods, Montauk.

*SPOROBOLUS CRYPTANDRUS* (Torr.) A. Gray.<sup>2</sup> Very rare. Bayville: dry sandy ridge just back of sea beach.

*MUHLENBERGIA SOBOLIFERA* (Muhl.) Trin. Rich hilly woods: Queens. Very rare. Only one colony of 3 or 4 plants. The writer can find no previous record for Long Island.

*BRACHYELYTRUM ERECTUM* (Schreb.) Beauv. Hilly rich woods; uncommon. Millneck; Smithtown; Port Washington; Queens.

*SPHENOPHOLIS OBTUSATA* (Michx.) Scribn. Meadow near salt marsh; rare. Merrick.

*PANICUM LONGIFOLIUM* Torr. Open swamp, bogs, and damp meadows; not uncommon. Bridgehampton; Manorville; Yaphank; Wyandanch; Rockville Centre; Hempstead.

*PANICUM AMARUM* Ell. Sea beaches; rare, but locally abundant at Eaton's Neck. Southold; Point O'Woods; Wading River; Bayville. In the first three localities the plants were few and scattered; at Bayville there were a few dense colonies of very large plants; at Eaton's Neck, back of the sand dunes, there were thousands of plants growing for a long distance.

*PANICUM BICKNELLII* Nash. Open sand, dry oak woods, and pine barrens; uncommon. Smithtown; Wantagh; Massapequa; Ronkonkoma; Hempstead Reservoir. Where the writer has found this plant it was represented by a single clump, except at the Hempstead Reservoir, where there is a colony of perhaps a dozen.

*PANICUM COMMUTATUM* Schult. Very rare. Laurelton, wet sand, edge of marsh; Cypress Hills, dry hilly woods.

*PANICUM WRIGHTIANUM* Scribn. Wet sandy shores, and damp grassy meadows; very rare. Sag Harbor, Long Pond; Bridgehampton, Poxabogue Pond.

*PANICUM LATIFOLIUM* L. Dry rich woods, and open scrub; not uncommon. Port Washington; Orient; Cypress Hills; Queens; Kew Gardens; Rockville Centre; Richmond Hill; Plattsdale; Forest Park.

## CYPERACEAE

*ELEOCHARIS ROBBINSII* Oakes. Shallow water, boggy shores, and bogs; uncommon. Watermill; Bridgehampton; Smithtown; Ronkonkoma; Sag Harbor.

*SCLERIA RETICULARIS* Poir. Wet sandy shores; rare. Bridgehampton, Poxabogue Pond; Sag Harbor, Long Pond.

*PSILOCARYA NITENS* (Vahl) Wood. Wet sandy shore; very rare. Sag Harbor, Long Pond.

*CAREX SHRIVERI* Britton. Swampy woods; very rare. Woodside.

<sup>2</sup> Reported by Homer D. House. Annotated list of the ferns and flowering plants of New York State. 1924.

*CAREX WILLDENOVII* Schk. Very rare. Greenport, open dry spot in swampy woods. (Previously reported from same locality by Roy Latham.)

*CAREX PALLESCENS* L. Bog; rare. Flushing. Only one clump seen.

*CAREX COLLINSII* Nutt. Very shady thickets in wet sphagnum; uncommon. Smithtown; Massapequa; Merrick; Wyandanch, two stations, one in hills and one in level pine barrens; Central Islip, in pine barrens.

*CAREX ANCEPS* Muhl. Rich hilly woods; not uncommon. Queens; Deer Park; Richmond Hill; Millneck; Kew Gardens; Roslyn; Cutchogue; Peconic. (The last two collected by Roy Latham.)

*CAREX LAEVI-VAGINATA* (Küken.) Mackenzie. Swamps; not at all uncommon on Long Island, about as plentiful as *Carex stipata*, and found in the same situations. The writer has more than once found them a few feet apart. Flushing; Meadowbrook; Setauket; Montauk; Rockville Centre; Millneck; Lakeview; Farmingdale; Wading River; Wyandanch.

### JUNCACEAE

*JUNCUS ARISTULATUS* Michx. Borders of bogs, swamps and marshes; rare. Matituck; Sag Harbor, Long Pond (2 stations). Determined by F. V. Coville.

*JUNCUS ARTICULATUS* L. Bogs, swamps, wet borders streams and ponds; uncommon. Seaford; Watermill; Montauk; Point O'Woods.

### HAEMODORACEAE

*GYROTHECA TINCTORIA* (Walt.) Salisb. Wet sandy shores; very rare. Sag Harbor, Long Pond.

### POLYGONACEAE

*TINIARIA CILINODIS* (Michx.) Small. Swampy open hilly woods; very rare. Cypress Hills, large colony. Determined by J. K. Small.

### CHENOPODIACEAE

*ATRIPLEX PATULA* L. Grassy roadside; rare. Hempstead. Determined by Paul C. Standley, who states: "a form of *Atriplex patula*, which in turn may be a variety of *Atriplex hastata*."

### DROSERACEAE

*DROSERA FILIFORMIS* Raf. Wet sandy shores and sandy bogs; uncommon. Sag Harbor, Long Pond; Bridgehampton; Montauk, Fort Pond; Manorville; Wading River, Long Pond.

### FABACEAE

*MEIBOMIA LAEVIGATA* (Nutt.) Kuntze. Dry hilly oak woods; very rare. Sag Harbor, Long Pond, one small colony. Determined by J. K. Small.

*FALCATA PITCHERI* (T. & G.) Kuntze. Swamps and moist thickets; rare. Cold Spring Harbor; Flushing.

### ELATINACEAE

*ELATINE MINIMA* (Nutt.) Fish. & Mey. Wet shores; uncommon. Determinations by M. L. Fernald. Montauk; Southold; Laurel. (Last two collected by Roy Latham.)

## VIOLACEAE

*VIOLA EMARGINATA* Le Conte. Dry oaks woods, plains, and pine barrens; uncommon. Hempstead Plains, at Hicksville and in Isle of Pines; Massapequa; Ronkonkoma; Plattsdale; Brentwood; Wyandanch; Central Islip. Mostly determined by Ezra Brainerd. (Intergrades with *V. sagittata* and *V. fimbriatula*.)

## ARALIACEAE

*ARALIA RACEMOSA* L. Hilly rich woods; uncommon. Plattsdale; Albertson; Queens; Wyandanch; Roslyn; Cold Spring Harbor.

*ARALIA HISPIDA* Vent. Dry or wet sandy ground; uncommon. Cutchogue; Flanders.

## AMMIACEAE

*SANICULA GREGARIA* Bicknell. Hilly rich woods; uncommon. Cold Spring Harbor; Richmond Hill; Millneck; Bayside. *Sanicula marylandica* and *Sanicula canadensis* are both widespread and common on Long Island.

*WASHINGTONIA CLAYTONI* (Michx.) Britton. Hilly rich woods. Not nearly as common as *Washingtonia longistylis*, and usually much smaller; plants one-half the size or but little more with rare exceptions.

*HYDROCOTYLE AMERICANA* L. Wet places in rich woods; uncommon. Millneck; East Hempstead; Plattsdale.

*HYDROCOTYLE UMBELLATA* L. Swamps and wet shores, mostly in water; rare. Montauk, Great Pond and Fort Pond; Sag Harbor, Long Pond; seen at Watermill.

*OXYPOLIS RIGIDIOR* (L.) Raf. Swamps; not uncommon. Massapequa; Valley Stream; East Meadowbrook.

## MONOTROPACEAE

*HYPOPITYS AMERICANA* (DC.) Small. Very rare. Very wet rich woods, south of Meadowbrook, one small colony. Determination by J. K. Small. The writer can find no previous record for Long Island.

*HYPOPITYS LANUGINOSA* (Michx.) Nutt. Dry hilly oak woods. Kings Park; Smithtown; Montauk, Hither Woods. Determinations by J. K. Small.

*HYPOPITYS INSIGNATA* Bicknell. Dry oak woods; not uncommon. Smithtown; Ronkonkoma; Sag Harbor; East Hempstead; Deer Park; Pinelawn; Central Park; Wyandanch. This species matures about six weeks later than the others.

## VACCINIACEAE

*VACCINIUM CAESARIENSE* Mackenzie. Swamp; rare. Ronkonkoma. Determination confirmed by P. C. Standley.

*OXYCOCCUS OXYCOCCUS* (L.) MacM.<sup>3</sup> Sphagnum bog, Ronkonkoma. Very rare.

<sup>3</sup> Norman Taylor, of the Brooklyn Botanic Garden, in a letter to the writer dated September 28, 1925, states: "Your collection of the small-fruited cranberry is the first to come from Long Island. E. S. Miller reported it from Wading River in the Bulletin of the Torrey Club 7: 18. 1880 and 10: 120. 1883. But his herbarium shows no specimens from that place."

It is of interest to record that in this same bog *Lycopodium carolinianum* was discovered by the writer a few years ago, not before known north of the New Jersey pine barrens, while *Oxycoccus Oxycoccus* is a plant of more northern or cooler climate than Long Island.

#### GENTIANACEAE

**BARTONIA PANICULATA** (Michx.) Robinson.<sup>4</sup> Damp or wet open places, and woods; widely distributed, but nowhere common. From some localities the writer found but one or two plants very variable. Determinations confirmed by M. L. Fernald, who writes: "*B. paniculata* behaves with you much as it does in eastern Massachusetts, but none of the specimens are so extreme as in Nova Scotia and Newfoundland." Montauk Point; Hempstead; Smithtown; Ronkonkoma; Flushing; Wyandanch; South Meadowbrook; Cold Spring Harbor; Millneck; Merrick; North Bellmore; Rockville Centre; Roslyn; Valley Stream. Some of the above plants were purplish-colored, but with no constant relation to any definite form. The plant found at Hempstead was found growing associated with *Bartonia virginica*, and both species were equally purplish-colored.

#### CUSCUTACEAE

On shrubs and herbs in swamps, bogs, swampy woods, and borders of ponds and streams. Determinations by T. G. Yuncker.

**CUSCUTA POLYGONORUM** Engelm. Very rare. Plattsdale, two colonies; Queens, woods. The writer can find no previous record for New York State.

**CUSCUTA CORYLI** Engelm. Sag Harbor. Very rare.

**CUSCUTA COMPACTA** Juss. Not uncommon. Valley Stream; South Meadowbrook; Laurel; Central Islip; Wyandanch; Smithtown; Flushing; Roslyn.

**CUSCUTA GRONOVII LATIFOLIA** Engelm.<sup>5</sup> Not uncommon. Sag Harbor; Valley Stream; Millneck; Watermill; South Meadowbrook; Montauk; Flushing.

**CUSCUTA GRONOVII VULGIVAGA** Engelm. Not uncommon. Queens; Valley Stream; Cold Spring Harbor.

#### LABIATAE

**CLINPODIUM VULGARE** L. Hilly rich woods; rare. Plattsdale; Wyandanch.

#### SCHROPHULARIACEAE

**GRATIOLA NEGLECTA** Torr.<sup>6</sup> (*Gratiola virginica* of Manuals). Wet borders of woods, swamps and ponds; uncommon. Flushing; Millneck; Plattsdale; Massapequa.

**ILYSANTHES DUBIA** (L.) Barnhart.<sup>6</sup> Swamps; rare. Plattsdale; Massapequa.

<sup>4</sup> Fernald, M. L. *Rhodora* 23: 149, 153, 157, 286-288. 1921.

Bicknell, E. P. *Bull. Torrey Club* 42: 32-33. 1915.

<sup>5</sup> In letter of October 16, 1925 to the writer, Professor Yuncker states: "It is often difficult to distinguish variety *latifolia* from *vulgivaga* of *Gronovii*. *Latifolia* has calyx lobes which reach, or almost reach, the corolla sinuses. The flowers are usually shorter than *vulgivaga* which has the calyx about half as long or less as the corolla tube. However, these two varieties merge one into the other so that it is difficult to keep them apart. In their extremes they are readily separable, but intergrading specimens are common."

<sup>6</sup> Pennell, F. W. *Torreya* 19: 143-152. 1919.

*ILYSANTHES INAEQUALIS* (Walt.) Pennell.<sup>6</sup> Swamps; not uncommon. Valley Stream; Plattsdale; Montauk; Cypress Hills; Calverton; Sweezytown; Central Islip, pine barrens.

#### LENTIBULARIACEAE

*SETISCAPELLA CLEISTOGAMA* (A. Gray) Barnhart. Wet sandy borders of ponds and streams, sandy swamps; rare. Sag Harbor, Long Pond; Central Islip, pine barren bog. In the last named locality the plant was growing where, earlier in the season in former years, *Setiscapella subulata* was collected by the writer in full flower. The writer had noted this same association at Long Pond, Wading River. For his comments on this, and for that of other field observers see Bull. Torrey Club 51: 198. 1924.

#### OROBANCHACEAE

*THALESIA UNIFLORA* (L.) Britton. Woods. Hempstead Reservoir; Plattsdale.

#### PLANTAGINACEAE

*PLANTAGO JUNCOIDES DECIPIENS* (Barneoud) Fernald.<sup>7</sup> Very rare. Grassy cliffs at sea beach, Montauk Point. Determined by M. L. Fernald.

*PLANTAGO OLIGANTHOS* Roem. & Schultes.<sup>8</sup> Salt marshes; not uncommon. Determined by M. L. Fernald. Cutchogue; Napeague; Freeport; Cold Spring Harbor.

#### RUBIACEAE

*DIODIA TERES* Walt. Open dry sand; not uncommon. Hempstead Plains, Garden City; Valley Stream; Springfield; Jamaica; Aqueduct; Sag Harbor.

#### LOBELIACEAE

*LOBELIA DORTMANNA* L. In shallow water; very rare in distribution, but plentiful where found. Watermill, Lake Nowedonah; North Sea, Big Fresh Pond.

#### COMPOSITAE

*HELENIUM AUTUMNALE* L. Swamps; rare. Valley Stream.

*EUPATORIUM PUBESCENS* Muhl. Dry meadow; very rare. Long Pond, Sag Harbor. Determined by J. K. Small.

*SOLIDAGO SPECIOSA* Nutt. Open field and dry open woods. Not uncommon but mostly in very small colonies, sometimes but two or three plants; locally abundant at Port Washington. Wyandanch; Southold; Albertson; Montauk; Orient; Millneck; Massapequa.

<sup>7</sup> Fernald, M. L. The maritime plantains of North America. *Rhodora* 27: 93-104. 1925.

<sup>8</sup> According to the writer's understanding both of the maritime plantains listed above have been combined as one species in the following works:

Britton & Brown's Illustrated Flora — *Plantago maritima* L.

Gray's Manual. 7th ed. — *Plantago decipiens* Barneoud

House's Annotated List of the Ferns and

Flowering Plants of New York State — *Plantago gibbosa* Raf.



# INDEX TO AMERICAN BOTANICAL LITERATURE

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Nuclear and cell division in *Nitella* and *Chara*<sup>1</sup>

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(WITH SIX TEXT FIGURES AND PLATES 10-13)

INTRODUCTION

The uncertain taxonomic position of the Characeae in the plant kingdom has made these plants the objects of interest to botanists for many decades; and by reason of their relatively simple and diagrammatically regular habit of growth as well as the ease with which they may be grown and handled under laboratory conditions, they have been the subject of numerous physiological, morphological, and cytological investigations. Notwithstanding these extensive studies, a review of the literature as to the general cytology of the group shows many differences in observation and interpretation concerning the presence of centrosomes, the method of formation of the spireme and chromosomes from the resting nucleus, cell plate formation, and the nature of the chromatic bodies in the cytoplasm about the nucleus in the resting condition and during mitosis. Most of the cytological work has referred to various species of the genus *Chara*, while comparatively little study has been devoted to *Nitella* and the other genera of the Characeae.

Nägeli (51), Schmitz (69), Treub (81), Strasburger (74) and Johow (31) were among the first investigators to describe the nuclei and nuclear and cell division in the Characeae. As early as 1844, Nägeli (51) described the nuclei of *Nitella flexilis*, *Chara vulgaris*, *C. hispida*, and *C. gracilis* as consisting of a clear, viscid, seldom granular nuclear vesicle and a nucleole. Schmitz (69, p. 367) states that the nuclei in *Chara* multiply in the same manner as the nuclei of higher plants, but he gives no drawings.

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Traub describes "nuclear fragmentation" and cell plate formation in cells of the antheridial filaments, one-celled antheridium, and terminal leaf cell of *Chara fragilis*. He finds that cell division is accomplished by the centrifugal growth of a cell plate instead of by furrowing or constriction. Shortly afterwards, Strasburger (74, pp. 194-196, *figs. 48-52. pl. 13*) figured spindles, chromosomes, daughter nuclei and cell plates in the nodal cells of *Chara foetida*. Jöhow rejects the views of the previous investigators, and maintains that nuclear and cell division in *Chara foetida* are in no way comparable to these processes in the higher plants. He describes the nuclear membrane as disappearing, leaving the chromatin bodies lying free in the cytoplasm where they begin to multiply. They later separate into two masses, and finally the small, granular bodies unite to form larger bodies. The membranes of the two daughter masses appear shortly afterwards, forming two nuclei. Nowhere in this process do spindle fibers appear, and the cell plate, arising between the daughter nuclei, appears in optical section as a double row of granules.

Zimmermann (88) believes that the nucleoli of the *Chara* nucleus fragment, pass out into the cytoplasm of the cell and are latter aggregated in the daughter nuclei. From his observation of the nuclei of unsectioned apical cells and eggs of *Chara aspera*, *C. jubata*, and *Nitella syncarpa*, Overton (57) believes that the nuclei possess no true nucleoli, but large, irregular chromatic bodies. In 1893 Schottländer (70) described centrosomes in the resting vegetative cells and in all stages of division in the antheridial cells of *Chara foetida*. In the resting stages two centrosomes are found lying on the nuclear membrane surrounded by a clear space, and as the nucleus goes into the spireme stage they begin to move apart. This continues until the centrosomes lie at the opposite poles of the nucleus.

Migula (44, pp. 52-53) describes indirect nuclear division in the apical and nodal cells of *Chara hispida*. In this species he claims to find more than one nucleus in each cell, but is not certain whether these bodies have arisen by direct or indirect division. Belajeff (4) describes the formation of chromosomes, spindles, and cell plates in the division of the antheridial filaments of both *Nitella* and *Chara*. He figures the chromosomes in *Chara* as small, round granules, and in *Nitella* as slender

filaments. He is not certain, however, about the presence of attraction spheres or centrosomes, but he describes small, deeply stained bodies lying on the nuclear membrane. The view of Schottländer as to the presence of centrosomes is confirmed by the observations of Kaiser (31) on dividing cells of three species of *Chara* and two species of *Nitella*. He finds centrosomes in the resting cells as well as in all stages of nuclear division. The centrosomes lie close to the nuclear membrane in pairs surrounded by a clear region, but no astral rays are figured.

The most complete account of mitosis in the Characeae is that of Debski (10, 11), who gives a considerable series of successive stages in the division of the nucleus and cell of *Chara fragilis*. As to the presence of centrosomes, Debski (10) was apparently uncertain, although in his summary he states that they are not present in the Characeae. In some preparations he saw centrosome-like bodies, but he was not sure whether they occur regularly. The first attempt at a cytological study of fertilization was by Götz (26). De Bary (9) had observed the entrance of the antherozoids into the oogonia several years before, but had not been able to see the behavior and fusion of the gamete nuclei in the egg. Götz figures dividing cells and nuclei in the development of the oogonium of *Nitella flexilis*, *N. opaca*, and *Chara foetida*, but he did not observe the presence of centrosomes.

Among the later cytological studies on the Characeae are those of Ernst (18), Oehlkers (55), Mirande (45), Riker (61), Mangenot (40) and Tuttle (82). Ernst has contributed much to our knowledge of the morphology and chromosome numbers of the dioecious, and, what he terms parthenogenetic forms of the Characeae. His observations will be referred to in more detail later. Oehlkers studied nuclear division in the germination of the oospore of *Chara fragilis*, *C. foetida*, and *Nitella syncarpa*, and holds that the reduction in chromosome number occurs in the first division of the nucleus of the germinating zygote, thereby confirming the claims of Debski (11) and Götz (26) that no reduction takes place at the time of the so-called gametogenesis. Directly opposed to this view are the observations of Tuttle, who maintains that reduction occurs in the first division of the cells which become the antheridia and oogonia. According to Tuttle, the *Nitella* and *Chara* plant as commonly seen in

nature belongs to the sporophyte rather than the gametophyte generation. The observations of Mirande, Riker, and Mangenot deal with mitochondria and plastids in the cells of Characeae, and will be considered in connection with these bodies.

#### MATERIALS AND METHODS

The following observations were made on plants of *Nitella gracilis* Smith, secured from one of the small ponds at Woods Hole, Massachusetts, *Chara coronata* Ziz. from Greenwood Lake, New Jersey, and an unidentified species each of *Chara* and *Nitella* growing in a shallow lake in Van Cortlandt Park, New York City. In an earlier paper (32) I have referred to the *Chara* species as *C. fragilis* Desv. because of its close resemblance to this form, but its identity has not been definitely established.

The plants were grown in tap water with sand loam as a substratum in large battery jars in north, south, and east windows of laboratories where the temperature was approximately 20° C. Vegetative growth in the battery jars was vigorous, some of the plants in the *Chara* cultures reaching a length of three feet. *Nitella gracilis*, *N. sp.*, and *Chara coronata* have fruited continuously in the laboratory for three years without regard to time of year. The unidentified species of *Chara* referred to as *C. fragilis* and reported by the author in an earlier paper as forming no reproductive organs under laboratory conditions from September, 1923, to May, 1924, has subsequently, 1924-1926, been found to produce a few antheridia and oogonia in winter under ordinary laboratory and greenhouse conditions. As reported, when the normal daylight period was supplemented with electric illumination, reproductive organs were produced in abundance during the winter months.

For the study of fixed material, Flemming's strong, medium, and weak chrom-acetic mixtures, Bouin's and Merkel's solutions, chrom-acetic acid, Allen's modification of Bouin's fluid, and Carnoy's, Benda's, Regaud's, and Němec's fluids were used as fixing agents. Merkel's and Flemming's solutions gave the least shrinkage and the best division figures. Heidenhain's iron-alum haematoxylin gave good results for the study of the chromosomes and the centrosome question, but Flemming's triple stain gave a finer differentiation of the nucleus and cell in general.

A careful comparison of the fixed and stained preparations

was made with cells mounted directly in water and sugar solutions, and with preparations stained *intra-vitam* with aceto-carmine, according to the method used by Belling (5) and Sands (66). The apical cells and the primordia of the antheridia and oogonia of *Nitella* are very favorable for such study before they become filled with chloroplasts and starch. Even more favorable are the cells of the antheridial filaments. These cells are free from chloroplasts and stored foods, and their relatively large nuclei are not obscured by strands of streaming cytoplasm and large vacuoles, such as are present in the stamen hairs of *Tradescantia* and the root tip cells of *Allium* and *Vicia*. Moreover, the entire processes of nuclear and cell division from the resting stage to the reorganization of the daughter nuclei and the formation of the new cell wall may often be seen in a single filament. A complete series of adjacent, successive stages of nuclear and cell division from the apex to the base, or vice versa, is not uncommon in the same filament. Frequently, long filaments may be found in which as many as fifty cells are undergoing division at the same time.

The method of preparing the living and aceto-carmine mounts of antheridial filaments was as follows: After removing the antheridia from the plants and mounting, the shield cells were peeled off with fine steel needles, exposing the manubria, capitula, and the filaments. The antheridial filaments were then carefully separated under the low power of the microscope with as little injury as possible. As soon as the cover glass was added the cells were ready for study.

The cells of the antheridial filaments are very sensitive to mechanical injury, and too much care cannot be used in selecting uninjured filaments for study. A slight injury to a cell will have a marked effect on the position and appearance of the nuclei in all cells of the filament. This effect is clearly shown in figure 51 of a filament injured a short distance from the apex. In this figure the nuclei have moved towards the cross wall nearest the injury. The nuclei in the cells near the apex lie against the basal cross wall, while those below the injury lie against the upper cross wall. Not only is the position of the nuclei modified by the injury, but their shape and appearance as well. The nuclei adjacent to the injured cell are very much flattened and seem denser and more compact in appearance. The change in ap-

pearance and position of the nuclei in this figure seems to be a general reaction of the nuclei of antheridial filaments to injury. Numerous long filaments have been found in which marked changes in shape, appearance, and position were visible in nuclei that were thirty cells removed from the injury.

This reaction of nuclei to injury (described for figure 51 of *Chara*) is wide-spread among plants and has long been known and figured extensively. Tangl (78), Nestler (54), Miehe (43), Schürhoff (71), Hottes (30) and Němec (53) observed that injury to a cell or group of cells in the roots and epidermis of various plants resulted in a general movement of the protoplasm as a whole towards the region of injury. Nestler found that the effect of injury to a cell could be detected by changes in the position of the nuclei in a radius of .7 to .8 mm. of the injury. The reaction is often so strong that the nuclei even pass through the cell wall nearest to the injury (Miehe, 43, *figs.* 2, 3, 4). Migration or extrusion of chromatin from the nuclei of pollen mother cells into the cytoplasm of adjacent cells in segments of anthers is a general occurrence, according to Körnicke (36), Farmer and Digby (19), Gregory (27), Digby (15, 16, 17), Rosenberg (62, 63), Gates (23), Fraser (22), Derschau (14), West and Lechmere (84) and Sinoto (73); and, although there is considerable controversy among these investigators as to the cause of this phenomenon, Körnicke and Sinoto (p. 109) believe that it is a direct response to certain traumatic stimuli, such as pressure and external injury to the anther.

#### NUCLEAR AND CELL DIVISION IN FIXED MATERIAL

##### *Resting nucleus*

In fixed and stained preparations the resting nuclei of the apical, nodal, and antheridial cells and eggs usually lie in the center of the cell, surrounded by the more or less homogeneous cytoplasm. The most striking feature of the nucleus of fixed preparations is the large nucleole-like body of highly stainable material at its center. This nucleole-like aggregate in *Nitella* and *Chara* appears to be similar to that described for fixed material of many of the Conjugatae, where the chromatin has been claimed to be aggregated into a compound nucleole-like body. In *Nitella* and *Chara* this body is large in proportion to the size of the nucleus, and in most cases appears to consist of a number



of smaller bodies. Frequently it lies in a large, clear space, but whether this is normal is questionable.

The small bodies making up the compound nucleole-like structure are often massed closely together, giving it an irregular outline and shape with light and dark regions throughout, as shown in figure 20. In some preparations where there has been slight plasmolysis the nucleole-like body is very dense and compact, but in properly fixed material the small bodies of which it seems to be composed may be entirely separate from each other. Many nuclei have been found in which the central region is filled with a large number of globular and angular, highly stained bodies. In the nucleus of an antheridial cell shown in figure 30 there are nine relatively large, round bodies and numerous smaller ones. Schottländer (*figure 36*) shows a similar nucleus of *Chara foetida* with five large bodies. In figures 29 and 31 a number of small bodies are grouped about a larger, central body. Figures 2, 3, and 5 show egg nuclei with large aggregated masses of deeply stained material in the center. In figures 2 and 3 a round body may be seen lying to one side of the mass. It appears vacuolated and has the appearance of a true nucleole. In figures 5 and 7, nuclei of an egg and apical cell, a peculiar modification is shown in which the compound mass has a number of knob-like projections. Figure 4 shows the nucleus of a nodal cell with four large round bodies in the center, and in figure 6, seven relatively small bodies are grouped in a clear central region of the nucleus. Strasburger (77, *figs. 2, 2b*) shows seven large, round bodies in nuclei of nodal cells of *Nitella syncarpa*. In addition to the mass of highly stainable material at the center, resting nuclei have been found with a large number of round and angular bodies in the nuclear network, such as are shown in figures 7 and 31.

Besides the granules and bodies described above, the resting nucleus shows a rather delicate chromatic reticulum or network. In most fixed preparations very little chromatin in the form of granules is visible in the reticulum. In the nuclei of figures 2, 3, 4, 5, 6, no definite granules are shown, but darker staining masses are visible in the reticulum, which appear as faint net-knots rather than definite bodies. Such a condition is not the universal rule, however. Many nuclei may be found with numerous dark staining granules and bodies in the nuclear network,

such as are shown in figures 7, 32, and 33. In the triple stain these bodies stain a faint violet in contrast to the deep red of the nucleole-like masses.

Whether the entire aggregate of highly stainable material at the center of resting nuclei in fixed preparations is to be regarded as a compound nucleole seems questionable. In many preparations, such as those shown in figures 2, 3, and 38, a round body that has all the appearances of a true nucleole may be found lying close to or in the center of the deeply stained mass of material. In such preparations the appearance is as though the chromatin material has aggregated into larger bodies around the nucleole. I have studied the same stages in unfixed and unstained and aceto-carmine preparations with the view of determining the nature of these chromatic masses. The nuclei of such preparations have a very different appearance from that shown in figures 1 to 7 and 29 to 34 of the fixed and stained material. No aggregate mass of bodies in the center is visible. Figure 8 shows an *intra-vitam* stained nucleus of an apical cell in which a single nucleole and numerous minute granules or bodies are visible, but no aggregate mass is present.

### *Prophase*

As the nucleus goes into the early prophase of division, an increase in size occurs, accompanied by a marked change in the appearance of the nucleus. In fixed preparations the delicate nuclear reticulum appears to gradually disappear, and the nucleus becomes filled with deeply stained, irregular strands and bodies. The strands are finally transformed into a thin, irregular, discontinuous spireme. The stages of this process are shown in figures 32 to 37. The nucleus shown in figure 33 has a few chromatin granules distributed in the nuclear reticulum in addition to two small nucleoles. Later stages are shown in figures 34 and 35, where short irregular bodies and strands of chromatin are shown in the nuclei. In figure 35 the nuclear reticulum has almost disappeared, and the nucleus seems to be filled with deeply stained angular bodies and strands of chromatin. The general appearance up to this stage suggests that the small net-knots and granules have increased in size and fused to form larger fragments and strands. A further concentration of the chromatin into strands and threads continues until the

condition shown in figures 36, 37, 39, and 40 is reached, where the nucleus is filled with an irregular, convoluted, discontinuous spireme.

The presence of the compound body of highly stainable material at the center of resting nuclei in many members of the Conjugatae has led investigators of this group to the view that the spireme and chromosomes come wholly or partly from the nucleole. Moll (49), Mitzkewitch (46), Karsten (34), Bergh (6), and Van Wissenlingh (83) describe the origin of the spireme and chromosomes of *Spirogyra* from the nucleole, and Merriman (41), in *Zygnema*, Wolfe (85), in *Nemalion*, and Golenkin (25), in *Sphaeroplea*, report a somewhat similar condition in these forms. This much debated question as to the possible origin of the chromosomes from a chromatin nucleole has also arisen in connection with the nuclei of *Nitella* and *Chara*. Riker (61) believes that part of the nucleolar content of the nuclei in *Chara fragilis* and *C. verrucosa* flows out into the spiral, spireme threads. If the bodies and granules aggregated in the center of the nucleus in fixed preparations are the chromatin granules fused to form larger bodies, this view is untenable. The spireme would then not arise from the nucleole but from the chromatin aggregated about it. I have been unable to find evidence in fixed and stained material for the formation of the spireme and chromosomes from the so-called compound nucleole, or the fragments and bodies in the nucleolar region. In the early prophase of fixed and stained preparations the nucleole-like body generally appears to loosen up. In figures 34 and 35 no large nucleole is visible, but a number of irregular fragments may be seen among the chromatin strands. On the other hand, the nucleole is still present in the late spireme stages shown in figures 9 and 12. A large number of nuclei similar to these have been found. In figure 11 a number of spireme threads are closely grouped around the nucleole, but do not appear to be arising from this body.

Figure 9 shows a nucleus in which the spireme has segmented and begun to thicken into chromosomes, and still a compound mass of deeply stained material is present. Somewhat similar conditions are figured by Debski (10, figs. 3, 4, 6) in *Chara fragilis*. However, it is apparent from the diminished size of the nucleole-like body in figures 10, 11, and 12 that it loses

some of its material during the prophases. As stated above, and shown again in figure 8, no such aggregated mass of material is visible in the resting nuclei of living cells and aceto-carmin preparations. In figure 8, a drawing from an aceto-carmin preparation of an apical cell, the nucleole appears as a round body, and the structure of the nucleus as a whole has a coarsely granular appearance. In prophase stages of aceto-carmin preparations corresponding to those shown in figures 95, 96, 97, and 98, a round nucleole is frequently to be seen. While the evidence from fixed and stained material that the spireme and chromosomes do not arise from an aggregated nucleole-like mass at the center of the nucleus is not altogether convincing, the conditions described in living material and aceto-carmin preparations, it seems to me, indicate that these structures arise from an irregular granular chromatin reticulum distributed more or less evenly in the resting nucleus. The condition as I find it in *Nitella* and *Chara* is similar in many respects to that described by Lutman (39) for *Closterium*.

In fixed and stained preparations the early spireme appears convoluted and discontinuous, and seems to completely fill the nucleus. Debski (10) describes a continuous spireme in *Chara*, but I have not been able to determine this for *Nitella* or *Chara*. In many nuclei of fixed preparations the spireme appears coarsely granular and irregular. Figures 9, 10, and 38 show irregular spireme filaments in nuclei of *Chara*, while in figure 11 of *Nitella* is shown a nucleus in which the spireme is relatively smooth and uniform in outline. This difference in appearance of the spireme in the two genera is not, however, as marked as in figures 9, 10, and 11. In the early stages of the spireme it is difficult to find split threads. In some preparations of *Chara* I have seen what appeared to be a clear central line in the spireme, but the longitudinal splitting cannot be recognized with certainty until the segments have shortened into chromosomes and passed into the equatorial plate and metaphase stages of division. However, figure 38 shows a nucleus in which a number of segments appear double or longitudinally split. The three segments in this nucleus that show a longitudinal split have also alternating thick and thin places that suggest the appearance of chromomeres. Several of the other segments in this nucleus show what look like vacuoles in a median position. This figure is in many

respects similar to one of Strasburger's (77, fig. 7) of *Nitella syncarpa*, in which he shows a number of split segments. Debski (10) was unable to determine the time at which the spireme segments split in *C. fragilis*, and believes that no preparation for the halving of the chromosomes is visible until metaphase. The segments of the spireme continue to thicken and shorten to form the definite chromosomes. In figure 12 is shown a late prophase nucleus of *N. gracilis* in which 32 segments are visible. The nucleole is still present, but relatively small in comparison with its size in resting nuclei. There is no question that in this nucleus it is a single, rounded body and not an aggregation of smaller ones. Figure 9 shows a nucleus of *Chara* sp. with a number of thick irregular segments. In this nucleus a dense mass of intensely stained material is still present, which is similar in many respects to the aggregated masses in resting nuclei.

In fixed material the cytoplasm surrounding the nucleus in the spireme stage often appears very dense and shows a more or less radial arrangement. In figure 11 is shown a cell in which this arrangement of the cytoplasm is faintly visible. On the other hand, such a condition is not clear in figure 12. Such a preparation as the one shown in figure 11 suggests that the spindle may arise from radially arranged strands of cytoplasm about the nucleus, as described for pollen- and spore-mother cells. In cells of the antheridial filaments I have observed the condition shown in figures 39 and 40. In figure 39 the cytoplasm is denser at the two poles of the nucleus, and in figure 40 faintly differentiated fibers may be seen. There is some evidence for the presence of polar caps in these cells, but it cannot be said with certainty from these preparations that this is the regular mode of origin of the spindle in *Nitella* and *Chara*. When the spindle is fully developed it is usually a broad diarch to bipolar structure. Its shape varies considerably in different cells. In the antheridial filaments it is generally broad in shape, as shown in figures 49 and 50, but often it may be barrel-shaped with the fibers converging to the two poles (FIGS. 41, 46, 47 and 48). In the cells of the antheridial filaments the whole spindle figure may lie parallel, diagonal or perpendicular to the long axis of the filament. In apical and nodal cells as well as those from which the antheridia and oogonia are developed, the spindle is generally barrel-shaped.

As previously noted in figure 38, I have observed what appeared to be a clear central line in the chromosomes of a few nuclei in late prophase of fixed material. When the chromosomes become arranged on the spindle the longitudinal split becomes more evident. This longitudinal split is shown in figures 16a and 16b. I have not been able to see this in living material and aceto-carmin preparations, although some of the chromosomes in the cells marked *A* and *B* in figure 105 show a light median line. However, I do not regard this as an actual split in the chromosomes, but rather as a high light effect caused by round objects when they are photographed. Seen in polar view in fixed preparations, the chromosomes are often well separated and can be counted with readiness. I have made numerous counts of twelve chromosomes in *Nitella* *sp.*, and the form of *N. gracilis* with which I have worked has 34. Figures 13, 14 and 15 of *N. gracilis* show polar views of chromosomes and an antheridial cell, nodal cell, and an antheridial filament cell. Figure 13 shows the one-cell stage of an antheridium mounted and stained *in toto* in aceto-carmin. In addition to the 34 chromosomes in this figure may be seen six granules in the cytoplasm on one side of the group and one on the opposite side. The number of chromosomes in *Chara coronata* and *C. sp.* has not yet been determined, but in numerous polar views of the latter I have counted as many as 40 chromosomes.

Comparatively little study has been given to the problem of chromosome number in the various species of Characeae. Ernst (18) reports 12 chromosomes in *Chara crinita*, *C. galioides*, and *C. aspera*, and 24 in *C. crinita* var. *bivalens*. Strasburger (77) finds 18 in *C. crinita* and *C. fragilis*, and 12 in *Nitella syncarpa*. In *C. foetida* Schottländer reports more than 19 chromosomes, Götz (26) finds 16 to 18, and Oehlkers (55), 16. Debski (11) and Oehlkers report 24 chromosomes in *C. fragilis*, but Riker (61) later finds only 16 in the same species. Oehlkers confirms Strasburger's (77) count of 12 chromosomes in *N. syncarpa*. It is evident from these conflicting reports either that the chromosome counts have been incorrect in many instances or that varieties and forms of the same species exist with different chromosome numbers. The latter view is highly plausible, since a large number of varieties and forms are found in species of *Nitella* and *Chara* that differ considerably in morphology.

My own observations on *N. gracilis* do not confirm the results of Tuttle (82) who finds a reduction in chromosome number occurring in the first division of the cells that become the antheridia and oogonia in the species of *Nitella* which he studied. Figure 13 shows a polar view of a division figure in an antheridium in the one-cell stage. While there appears to be some evidence of pairing of the 34 chromosomes in this figure, no reduction occurs in this division, since the same number of chromosomes may be found in the cells of the antheridial filaments (FIG. 15). According to Tuttle's view, the division shown in figure 13 is the reduction division. Figure 15 shows 34 chromosomes in a cell of an antheridial filament consisting of approximately 30 cells. At least 36 divisions intervene between this polar view and that shown in figure 13, but no reduction in chromosome number has occurred. Figure 14 shows 34 chromosomes in a polar view of a division in a nodal cell. According to Tuttle's view that the *Nitella* plant as commonly seen growing in nature belongs to the sporophyte generation, we should expect to find in this figure double the number of chromosomes present in figure 15 of an antheridial filament. I have likewise counted 34 chromosomes in polar views of divisions in the 4, 8, and 16-cell stages of antheridia.

The chromosomes in the species I have studied vary considerably in size. They appear as long and short rods, wide V's and U's, and hook-shaped figures, as shown in figure 14 of *N. gracilis*. It is difficult if not impossible to classify them according to size. In figure 14 there are approximately 14 small, 15 intermediate, and 5 large chromosomes. Such a classification, however, can only be approximate, since the variation in size is so gradual. The large chromosomes labeled *o* in figure 14 can readily be recognized in the divisions of the vegetative cells. In figure 17 they are very conspicuous in the anaphase group of chromosomes. In *Nitella sp.* there are ten long approximately equal and two short chromosomes. As shown in the cell marked *A* in figure 105, the longest chromosomes in the equatorial plate stage may extend from the equatorial region almost to the ends of the cell.

An unusual variation in the size of the chromosomes in different cells has frequently been noted in the antheridial filaments of *Chara sp.* In numerous long filaments the chromosomes

have all appeared as round bodies or very thick rods more or less uniform in size, while in other filaments of the same antheridium they have shown the same variation in size and shape shown in figure 42. I have observed this condition both in fixed and aceto-carminic preparations. Figure 104 shows the chromosomes as round bodies grouped in the center of the cells. In the division figures shown in figures 106 and 110 the chromosomes are considerably longer and more variable in size. This contrast is further shown in figures 42 and 43. In figures 42, 44, 45, 46 and 47, the chromosomes are variously shaped rods, while in figure 43 they are scarcely twice as long as round. I have not observed such a marked difference between the chromosomes in various cells of the antheridial filaments of *N. gracilis*, however, but the chromosomes shown in figure 15 are very short, thick rods in contrast to the various sizes and shapes present in figures 41 and 46.

Belajeff (4) describes the chromosomes of *Nitella* as slender filaments, and those in the antheridial filaments of *Chara* as round granules. With the exception of such cells as those shown in figures 40 and 104, the chromosomes of my preparations of *Nitella* and *Chara* are of various sizes and shapes. Debski (10) has figured the chromosomes of *C. fragilis* as slender and long drawn out. The figures of Kaiser (32) are too small to show the relative shape and sizes of the chromosomes of the forms which he studied. Strasburger (75) describes the chromosomes of *C. fragilis* as long, variously-shaped structures that are closely grouped together. Ernst (18) reports that the chromosomes in the antheridial filament cells of *C. crinita* are somewhat longer than those in the vegetative cells.

#### *Metaphase, anaphase, telophase*

In the metaphase of fixed and stained preparations the chromosomes are arranged in the manner shown in figures 16a, 16b, and 46. In antheridial filaments the equatorial plate may extend completely across the cell. In most cases the chromosomes of such cells are too numerous and large to find space in the short diameter of the cell. The equatorial plate may then lie in a diagonal position, and not infrequently parallel to the long axis of the cell, as shown in figures 44 and 106. In metaphase the so-called split of the chromosomes becomes evident.



In figures 16a and 16b are shown chromosomes with a definite longitudinal split. The split appears to begin in a median or sub-median position and extends gradually to the ends of the chromosomes. The halves generally move apart in the shape of V's and U's, the ends remaining longest in contact. In figures 45 and 46, numerous wide V's and U's are shown. The shape of the chromosomes in late metaphase indicates a median and sub-median fiber attachment, but numerous cells have been found where the fiber attachment to the chromosomes is atelomitic and telomitic as well. In figure 17, the large chromosomes are hook-shaped at one end, suggesting the atelomitic attachment.

As the chromosomes move towards the poles in the anaphase, they are generally V, U, and hook-shaped, with the bend towards the poles of the spindle. This is shown in figures 17, 18, and 47. In cells of the antheridial filaments where the equatorial plate lies diagonally in the cell, as early observed by Treub (81) and Giesenhagen (24), and shown in figures 41, 110, and 111 of my preparations, or parallel to the long axis of the cell, as in figures 44 and 106, the spindle and chromosomes apparently rotate in the cell during anaphase and early telophase, so that the cell wall which is to be formed later between the daughter nuclei will divide the cell transversely. In cells where the equatorial plate lies approximately parallel to the long axis of the cell, as in figures 44 and 106, the rotation must of course be approximately 90 degrees in order that a transverse wall may be formed. It sometimes happens that the rotation of the division figure is not completed before the daughter nuclei are formed and the new cell wall begun. In such cases a corresponding modification of the position of the new wall follows. The separation of the chromosomes and their movement to the poles in *Nitella gracilis* is generally regular, but occasionally "lagging" chromosomes are visible on the spindle (FIG. 18).

The fibers which appear attached to the chromosomes in fixed and stained preparations are very delicate but distinct. The central spindle fibers, however, are not very distinct until late anaphase and telophase. In these stages the central spindle fibers extend the entire length of the spindle and often appear in groups. The spindle at this time is very broad in the equatorial

region and markedly barrel-shaped. Frequently the poles of the spindle converge towards bodies of deeply stained material, as shown in figure 17.

In late anaphase the chromosomes are generally well separated and easily distinguished (FIGS. 18, 47, 48), but in early telophase, with the appearance of the nuclear membrane, they begin to lose their individual identity. The dispireme stage is well marked in figure 19. The chromatic strands at first appear to be more or less even and homogeneous, but in later stages light regions appear in the threads, giving a vacuolated appearance. About the time of the appearance of the nuclear membranes around the daughter nuclei, large, round, red-staining bodies are visible in the nuclei, which have the appearance of nucleoles. In the upper of the two nuclei shown in figure 19, a single body is present. In figures 48 and 49, the nucleoles are shown after the dispireme has begun to break up. There is in these preparations no evidence of the aggregation of stainable material about the nucleole, so characteristic of the resting nuclei in figures 1-8. The dispireme disappears shortly, and the daughter nuclei pass into the condition shown in figures 48 and 49. Following this they take on the characteristic resting appearance. Scattered chromatic bodies appear at the stage shown in figure 50. Later an aggregated body of deeply stainable material appears (FIGS. 20, 21).

#### *Cell plate and wall formation*

The formation of the cell plate in *Nitella* and *Chara* begins late in the division stages, and the thickenings in the spindle fibers in most cases do not appear until the daughter nuclei are completely organized. These thickenings, in fixed and stained preparations, are formed in the equator of the spindle and appear to be swellings on the central spindle fibers. In many cells, the equatorial region of the spindle may be filled with irregular or round deeply stained granules (FIGS. 19, 27) of various shapes and sizes. These granules do not appear to be connected with cell plate formation, since, in my preparations very few of them are present in the equatorial region where and at the time the spindle fibers have begun to thicken. On the other hand, Zacharias (86) believes they are the cell plate elements, and Johow (31) claims that a row of these granules

is transformed directly into the cell plate. In figure 20, the thickening in the fibers is visible across the whole spindle. The process of thickening appears to be accompanied by a shortening of the fibers, as though the material of which they are composed were going into the cell plate. The plate becomes visible in the central region of the spindle first, and is apparently developed outward toward the periphery. In figure 21, the cell plate is visible in the center of the spindle but does not extend to the plasma membrane of the cell. While the spindle fibers are shortening, clear regions free from fibers of any kind appear between them and the daughter nuclei. In fixed preparations there appears to be no so-called granular "trophoplasm" such as is described by Timberlake (79) for *Larix* in this region. With the growth of the cell plate toward the periphery and the shortening of the fibers, the daughter nuclei move nearer the equatorial region (FIG. 21) and often lie very close to the young cell plate.

The cell plate appears to grow from the central region toward the periphery of the spindle. At the time the plate appears in the central region, there are no visible thickenings in the peripheral fibers of the spindle. Figure 21 shows an early stage of cell plate formation in which it is present only in the center of the spindle and does not extend to the periphery of the spindle. The fibers beyond the ends of the cell plate are very numerous and dense and extend to the plasma membrane. It appears from this figure as though additional fibers have arisen in the cytoplasm at the periphery of the spindle to complete the growth of the cell plate, but whether this has occurred could not be determined with certainty from such preparations. Possibly these are the same fibers that appear to cross each other at the periphery of the spindle shown in figure 18. Figure 22 shows a later stage in which the cell plate has reached the plasma membrane. The successive stages in cell plate formation are more difficult to follow in cells of the antheridial filaments, due to their small size, but the process is the same here as in vegetative cells. In figure 49 two cells are shown in which the cell plate extends entirely across the broad spindle to the sides of the cell. Figure 50 shows a later stage where the plate is denser and thicker than in the preceding figure and appears to be fully formed.

*Centrosomes*

Owing to the fact that centrosomes have been described in various *Characeae* by Schottländer (70) and Kaiser (32), particular attention has been given to all kinds of granules and bodies lying near the nucleus and in the cytoplasm during each stage in mitosis, but no structures have been found that resemble or behave like the centrosomes of animal, algal, and fungus cells. In the resting condition, as well as in division, the nucleus is often surrounded by numerous granules of various sizes and shapes. Their size varies from that of a nucleole to a point where they are scarcely visible. Generally these granules or bodies stain like the nucleole and chromosomes.

These bodies often lie in contact with or near the nuclear membrane in a clear unstained region. In the resting cell they may be found in any region near or in contact with the nuclear membrane. In cases where they occupy such a position and lie in a clear space, they might easily be mistaken for centrosomes. Figure 1 shows a number of granules near and in contact with the nuclear membrane. In the cell here shown, two of the granules are approximately equal in size and surrounded by a clear space. They appear as two centrosomes, resulting from a recent division, which have begun to separate. In figure 11 may be seen two bodies similar in size near the nuclear membrane but further separated than those in figure 1. Figures 16a, 16b and 17 show cells in which a number of granules lie at or near each pole of the spindle. In figure 17, the poles of the spindle converge towards two large irregular bodies. This figure resembles those of Debski (10, *figs.* 13, 14, 16, 25) in which he shows a number of spindles whose poles are somewhat centered on deeply stained bodies.

It was doubtless the appearance of such granules near or in contact with the nuclear membrane that led Schottländer and Kaiser to claim the presence of centrosomes in the *Characeae*. The study of hundreds of cells has convinced the writer that these granules are of the same nature as the larger bodies lying scattered in the cytoplasm, and differing from the latter only in size and position. In no instances have astral rays or fibers been seen in connection with them.

*Chromatic granules in the cytoplasm*

No adequate description, it seems to me, has been given of the nature, origin, and distribution of the deeply staining granules and bodies of various sizes and shapes in the cytoplasm of Characeae. Kaiser (32) regards them as equivalent to Altmann's granules because of their staining reaction. Zimmermann (88), Migula (44), and Debski (11) believe they are extruded into the cytoplasm from the nucleus, and more specifically from the fragmenting nucleole. Götz (26) holds that the granules in the eggs are extruded from the egg nucleus shortly before fertilization. Riker (61) studied the granules in dividing cells of *Chara*, and believes that they originate from the nucleole, but are not included in the chromosomes and daughter nuclei when they are formed. He calls them prochondriosomes and holds that they first appear in the equatorial region of the spindle. From this position they migrate outward into the cytoplasm around the spindle and become chondriomes. Mirande (45) found corpuscle-like bodies in the cytoplasm of *Chara* which continue to multiply during mitosis and which have a staining reaction like nucleoli. He thinks that they have in part been extruded from the nucleus. In addition, Mirande describes chondriomes near the nucleus which migrate to the periphery of the cell, develop central vacuoles, and become chloroplasts.

Mangenot (40) made an extensive study of the constituents of the cytoplasm in cells of *Chara fragilis* to determine the origin of the plastids and starch grains. He found that the cells are filled with long rod-shaped chondriosomes, and, in addition, around the periphery of the apical cell there is a continuous border of rounded and oval bodies each with a small region in the center, which gives the reaction of starch with iodine. In the young eggs, there is first a retrogression of the plastids until they are indistinguishable from the chondriosomes. The chondriosomes then elongate into chondrioconts and form a vesicle in the center, which stains like starch. These starch-containing chondrioconts rapidly increase in size until the whole egg appears filled with large starch grains, according to Mangenot. He describes a similar retrogression of the plastids in the antheridial cell, until they have completely disappeared.

A careful study was made of these chromatic granules to determine their origin, staining reaction and general distribution in successive mitoses, and their relation to the nucleus, chondriosomes, and plastids. Material fixed with mitochondrial fixatives was carefully compared and checked up with preparations killed in Flemming's, Merkel's, Bouin's, and chrom-acetic solutions. In *Nitella* and *Chara*, the chromatic granules are as numerous in the resting cells as in cells undergoing division. Figure 23 shows a resting cell of *N. gracilis* whose upper part, marked *x* in the figure, was almost filled with densely stained granules. The total volume of these granules in the cytoplasm appears to be almost as large as the volume of the entire nucleus. Strasburger (77, *fig. 3*) and Debski (10, *figs. 1, 2, 8, 9*) figure similar bodies in resting cells. However, they are inclined to think that the bodies are most numerous during mitosis, while Riker (61) believes they have their origin in the nuclear division stages, and does not figure them in resting cells. Some resting cells may be entirely free from granules, while other cells may have as many as a hundred densely stained bodies or granules. This condition is true of dividing cells as well. Figure 12 is a section of a cell in the late spireme stage in which there is but one granule in the cytoplasm. Figure 18 shows a cell in late anaphase with only a few granules present, while in figure 19 of a telophase, the upper nucleus is almost obscured by bodies of all sizes and shapes.

The distribution of the chromatic granules in cytokinesis, though irregular, is, nevertheless, characteristic. In apical cells and those that are to become the antheridia and oogonia, the resting nucleus frequently lies nearer the basal wall than the center of the cell, and the chromatic granules are generally more numerous in the upper part of the cell. They often lie in a somewhat semicircular group near, and sometimes in contact with the nuclear membrane, and in the cytoplasm of the upper part of the cell, as shown in figure 23. Accordingly, when the apical cell divides, it appears as if a greater number of granules is distributed to the upper of the two cells that result from the division. Very often the upper pole of the spindle of dividing apical cells is surrounded by numerous deeply stained bodies or granules. Generally the cell which is destined to form the node receives fewer granules from this division of the apical

cell; yet many young nodal cells have been found with as many as forty chromatic bodies in the cytoplasm. In the first four divisions of the antheridia the distribution of granules appears to be towards the external periphery of the cells. Figure 17 represents the horizontal division of an antheridium in which are shown numerous bodies scattered in the cytoplasm of the external peripheral part, marked  $x$ , of the cell; that is, the part of the cell nearest the periphery of the antheridium. In figure 19, a telophase of the horizontal division of an antheridium, approximately 30 bodies of various sizes are gathered about the upper of the daughter nuclei, while no granules are present in the lower part of the cell. Figures 16a and 16b show metaphases of the fourth division of an antheridium which divides the octants into inner and outer cells. Practically all of the granules lie at the outer pole, marked  $x$ , of the spindle, and it is likely that they will be included in the outer cell when division is complete. In the subsequent divisions of the antheridia, the chromatic bodies seem to disappear gradually. When the shield cells, manubria and capitula are well differentiated, and the cells of the antheridial filaments fully formed, very few and often no granules are to be seen in the cytoplasm of the latter cells.

The distribution of the chromatic granules in the cell which becomes the egg, after the so-called 'Wendungszellen' have been formed, appears very much the same as described in the apical cell. When the first 'Wendungszelle' is cut off tangentially from the egg cell, relatively few bodies are included in it. They are generally found in greater numbers in the egg, often distributed in the manner shown in figure 24. In some cases when the first 'Wendungszelle' is cut off apically, it generally receives more granules than when formed tangentially. This is not true of all cases, however. Very few chromatic bodies appear to be included in the second and third 'Wendungszellen' in *Nitella gracilis*. In figure 25 is shown an egg with the first and second 'Wendungszellen,' in which a large number of round and irregular bodies are present in the apical portion of the egg, while comparatively few are to be seen in the 'Wendungszellen.' When the egg is fully developed, the cytoplasm may often contain as many as 50 densely stained granules. Later, at the time of fertilization, a large number of granules is often aggregated near the apex of the egg.

The distribution of the chromatic bodies on the mitotic figure varies. They are frequently found extending in a row across the equatorial plate of the spindle, as illustrated in figures 19 and 27. In figure 19, granules are also present at the upper pole of the spindle as well. In figure 27, the deeply stained bodies on the spindle appear to be almost equal in volume to one of the groups of daughter chromosomes. The presence of granules in one place on the spindle does not exclude their presence at other places. At the same time that they appear on the spindle, they may be seen in great numbers in the cytoplasm. Often in early telophase, as shown in figure 19, the chromatic bodies may sometimes be so numerous around the poles of the spindles that the daughter groups of chromosomes are almost obscured. In figure 28 is shown an equatorial plate stage in which an unusually large number of granules and fragments are present at both poles of the spindle. Many cell division figures have been found in which the poles of the spindle converge towards a granule or a group of granules, as shown in figure 17. Where single granules lie in such positions, they may easily be mistaken for centrosomes. Many mitotic figures, on the other hand, may be found in which no granules whatever occur. Such a cell is shown in figure 18.

Concerning the size, shape and staining reaction of the chromatic granules, considerable variation has been found. Their size varies from that shown in figures 16a, 27 and 28 to a point at which they are scarcely visible. Some of the large, round granules are 3.5 microns in diameter, and I have found a few single bodies as much as 5 microns long and 2 microns in diameter. The bodies may be oblong, round or angular in shape; they may occur singly or in groups. In figure 23 may be seen several angular aggregated bodies in addition to the single ones, and it is apparent in this and many other similar preparations that such bodies are made up of smaller granules, so that they are irregular in outline. Their reaction to stains is generally the same as that of the nucleole. In Flemming's triple stain they may be deep red to faint violet; in haematoxylin, deep blue to black, and in aceto-carmine and safranin, red.

A careful study of the granules with the mitochondrial fixative was made to determine their relationship to the chondriosomes described for *Chara* by Riker, Mangelot and Mirande.



Benda's, Němec's and Regaud's fixatives were used. The best results were secured with Benda's and Regaud's fluids. In cells fixed in these fluids and stained with haematoxylin and crystal violet-sulpharizarin, according to Benda's method, the chondriosomes are abundant, particularly near the nuclear membrane and the periphery of the cell. In addition to the chondriosomes in the cytoplasm of such cells may be found large round, oblong, and often angular-shaped bodies, which are different in size and shape from the chondriosomes. Such bodies are similar in every respect to the granules occurring in cells fixed in strong chrom-acetic solutions, Flemming's, Bouin's and Carnoy's fluids. Figure 26 shows a cell in which these granules are sharply contrasted with the chondriosomes. A comparison of this cell with those shown in figures 1, 17, 23 and 24, shows the similarity in size, shape, and general appearance of these granules in cells fixed in entirely different killing agents. There is no distinct border line of size in figure 26, however, between these granules and the chondriosomes. The smallest granules and the large chondriosomes are almost indistinguishable in size and shape, and it is impossible to differentiate the one from the other with certainty where they are so similar. The large granules, on the other hand, can easily be distinguished from the chondriosomes by size, shape, staining reaction, and general appearance. They appear to be much the same regardless of the kind of fixative used in killing the cells. Riker's contention that they are easily destroyed in strong fixing agents has not been confirmed from my preparations. In fixing fluids in which chondriosomes have been reported to be generally dissolved, the size, shape and distribution of the granules is essentially unaffected. They are as numerous in cells fixed in strong chrom-acetic solutions as in cells killed in fixatives lacking acetic acid. From these observations it appears to me that the large granules or bodies in the cytoplasm of cells of *Nitella* and *Chara* are distinctly different from the chondriosomes and should not be classified as such.

#### NUCLEAR AND CYTOPLASMIC CHANGES IN WATER AND SUGAR SOLUTIONS

I have tested quite fully the effect of aceto-carmin on the appearance of the so-called resting nucleus and the cytoplasm

in the antheridial filaments of *Nitella* and *Chara*. The stock solution of aceto-carmin, made up according to Belling's formula, was diluted with once-distilled water to the desired strength. A dilution of one part of aceto-carmin to eight parts of once-distilled water gave good results, and all of the cells shown in PLATE 13 were studied in this dilution. For comparison I have also studied and photographed these resting nuclei in tap and distilled water and in sugar solutions of various strengths. The cells of the antheridial filaments undergo a marked change in appearance within a few minutes after the filaments are teased out in the water, and similar, but less rapid, changes occur when they are mounted in sugar solutions.

These changes in the appearance of the nucleus and cytoplasm when the cells are mounted in water and sugar solutions take place with striking rapidity, as is shown in the figures in PLATE 12. As a means of presenting these changes as objectively as possible, I have photographed the cells at different specified intervals. These figures (PLATES 12, 13) illustrate the difficulties and weakness of photographic methods when used on cytological problems, but none the less, in view of our present interest in the mass appearances of protoplasm as bearing on the problem of its colloidal make-up, they serve as an excellent check and supplement to drawings of fixed and stained preparations.

Figures 52 to 57, and 58 to 61 are photographs of filaments mounted in tap water. The first series shows four nuclei in the same filament photographed at successive periods after mounting. In figure 52, photographed ten minutes after teasing out in water, the outlines of the nuclei are quite sharp as compared with nuclei when first mounted (FIG. 68). Several minutes are always required in mounting, locating, and photographing the filaments, and the appearance of the nucleus, in the meantime, undergoes a marked change. In the procedure which I have followed, from five to fifteen minutes elapse between the time when the antheridia are first broken open in the water and the time when the exposure is made.

The nuclei when first mounted appear comparatively homogeneous, but highly refractive, with very faint outlines. Figure 68 shows seven cells as little modified by treatment with water as any I have been able to secure in photographs. The conspicuous dark spots in this photograph are due to extraneous material

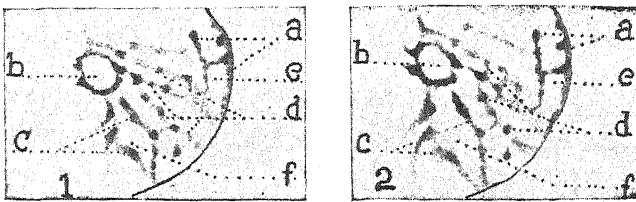
outside the filaments and are to be disregarded. In these cells very much the same appearance characterizes both the nucleus and cytoplasm, and little structural differentiation is visible. I regard the appearance of these cells as being very nearly that of the so-called resting nucleus unmodified by treatment, and for comparison I shall use these cells as the starting point in describing the nuclear and cytoplasmic changes in the series in PLATE 12.

Whether the nucleus in these cells is a two phase or poly-phase colloidal system is not obvious from the photograph, but it is, nevertheless, evident that if colloidal phases are present in the nucleus, their indices of refraction are very much the same, and, consequently, show little visible differentiation. From the photograph the nucleus might be a molecularly dispersed solution of protein in nuclear sap, or a hyaline, optically almost homogeneous, emulsoid gel.

In figure 52, taken ten minutes after mounting in tap water, the nuclear outlines are much more conspicuous and their content is optically more heterogeneous than in figure 68. The general appearance of the nucleus perhaps suggests that it is made up of a more hyaline ground substance with the darker elements imbedded in it. There is, of course, no adequate evidence from such a figure that the clearer hyaline material is a watery nuclear sap in which dense chromatic elements are distributed, but the general appearance, none the less, suggests a confirmation of the conclusions as to the nuclear make-up which have been reached by a study of fixed and stained preparations. In the uppermost of the four nuclei shown in this figure, the dark elements appear to consist of rather rounded granules and more angular masses arranged in short strands and groups. With the view of bringing out more clearly what seems to me to be the appearance and arrangement of the light and dark elements in the series of photographs of the uppermost nucleus in figures 52 to 58, I have made a corresponding series of diagrams of a sector of this nucleus. In the region of the nucleus shown in text-figure 1, there are a number of short strands that appear to be made up of small irregularly-shaped bodies and granules. Next to the nuclear boundary is a nodular strand that extends a considerable distance around the nuclear periphery. Although more or less uniformly dark, its irregularity

suggests that it may be a connected series of bodies and granules. Parallel to it but slightly removed from the nuclear boundary is another perhaps similar strand which is likewise nodular in outline. These two strands designated by *a* in the text-figure appear to be connected in the center by a short, thin strand. The light region between these two strands is labelled *e* in the text-figure.

In a somewhat eccentric position in the nucleus may be seen a highly refractive rounded body, which is evidently the nucleole of fixed and stained preparations. Below the nucleole is the suggestion of two somewhat parallel, interrupted strands with an elongated light area, *f*, between them. These two strands, *c* in the text-figure, are not uniformly dark, and give the impression



TEXT-FIG. 1. Diagram of a portion of the uppermost nucleus of FIG. 52, PLATE 12. FIG. 2. Diagram of a portion of the uppermost nucleus of FIG. 53.

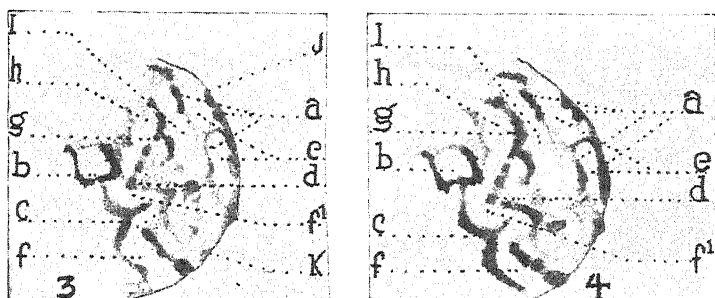
that they consist of more or less connected irregular granules. The relative light and dark appearance of these elements may be due to certain of them being more or less out of focus. There is a further suggestion of strands of connected granules in the region labeled *d* in the diagram. These strands appear to be somewhat radially placed around the nucleole.

As noted above, the general appearance of the nucleus suggests that the light hyaline areas make up a ground substance or continuum in which the dark elements are dispersed. The appearance is somewhat that of an emulsoid with the light elements constituting the medium of dispersion and the dark elements the dispersed aggregates. However, the presence of irregular strand-like elements made up of granules and bodies more or less connected suggests an interrupted reticulum suspended, perhaps, in an optically more homogeneous medium.

Figure 53 shows the same segment of the antheridial fila-

ment photographed five minutes later. Very little change is perceptible. The same elements are recognizable in the uppermost nucleus and in the same arrangement as they are shown in figure 52. The dark elements perhaps seem darker and denser. It is possible, however, that this may be due to a slight difference in exposure and development of the negative. Text-figure 2 is a diagram of this same nuclear region from figure 53. The same elements shown in text-figure 1 are recognizable throughout.

Figure 54 shows the same filament photographed twenty minutes after mounting. There is here a more marked change



TEXT-FIG. 3. Diagram of a portion of the uppermost nucleus of FIG. 54, PLATE 12. FIG. 4. Diagram of a portion of the uppermost nucleus of FIG. 55.

in the nuclear make-up than in figures 52 and 53. If we compare the appearance of the uppermost nucleus in this photograph with its appearance in figures 52 and 53, we note many differences in the nuclear elements and their arrangement. It is, of course, possible that these differences are due to a difference in the focal plane shown in figure 54. I have made a diagram of the corresponding sector of this nucleus for this figure, which is shown in text-figure 3. The black spot that is so conspicuous above and to the right of the nucleole is due to extraneous material outside of the nucleus and may be disregarded. The nucleole still appears as a highly refractive body, but its outline, due to the dark elements surrounding it, appears to be somewhat angular. Below the nucleole in the region occupied by the two strands labeled *c* in the two previous text-figures is a thick, dark strand-like element, *c* in text-figure 3, that can be traced from the nucleole to the periphery of the nucleus. Immediately

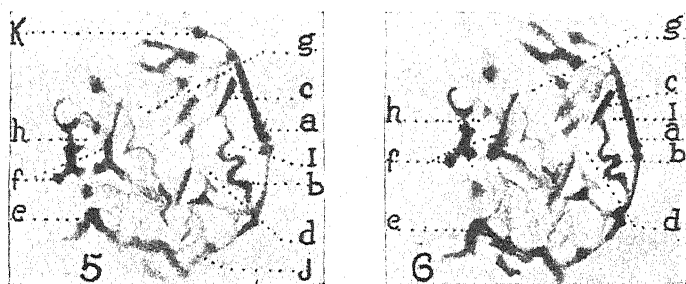
to the right of the nucleole is another dark element, *d*, somewhat zigzag in outline. Between these two strands a hyaline zone extends from the region labeled *h* through *f*<sup>1</sup> and *f* to the nuclear boundary. In the region marked *g* is a shorter, denser strand, and adjacent to the nuclear boundary, as in the two previous figures, is a dark irregular strand continuing from *j* to *k*. Somewhat parallel to it is another strand, *a*, and between them is an elongated hyaline area marked *e*. These two strands are very similar in position and appearance to the strands labeled *a* in text-figures 1 and 2. Between the elements *a* and *g* is another hyaline zone, marked *i*. In figure 54 the dark elements appear less like a series of connected granules and bodies than in figures 52 and 53.

The photograph shown in figure 55 was taken of the same filament twenty-five minutes after mounting. The uppermost nucleus of this figure has much the same appearance as in figure 54. The figure appears perhaps to show more contrast, and this again may be only a matter of variation in the photographing. I have, however, made a text-figure (4) of the corresponding sector of the uppermost nucleus in this figure to bring out the striking similarity in size, shape, and position of the nuclear elements as compared with those in the picture taken five minutes earlier.

Figure 56 shows a photograph of the same segment of the filament taken thirty minutes after mounting. In the uppermost nucleus very little is to be seen of the elements shown in figures 54 and 55. In this figure there is very little, if any, suggestion of granules or bodies arranged in series and groups, such as appear in figures 52 and 53. The nuclei are somewhat less in diameter, and the whole effect is somewhat that of being out of focus. The appearance of the nucleole, however, indicates that the focal plane cannot be very different, and figure 57 agrees well in general appearance with figure 56. Text-figure 5 is a diagram of the corresponding sector of the uppermost nucleus. Lying slightly over the highly refractive nucleole, *h*, may be seen two short, dense, branched bodies labeled *f*. Extending half-way around the periphery of the nucleus from *k* to *j* in text-figure 5 is a long irregular strand of varying density. In the portion marked *a*, it appears very dense and straight, and projecting downwards at an acute angle from this portion

of the peripheral strand is a short, thick element marked *c*. Below this is a conspicuous undulating strand labeled *b*. Between it and the peripheral strand is the elongated hyaline area, *i*. In the regions of the nucleus marked *g* and *d* are two conspicuous rather broad hyaline zones which may be continuous with each other. Below the region occupied by the nucleole is a long, dense, irregular strand marked *e*. In addition to these dense strands are other fine, lighter and less dense strands which appear to be connected with the denser elements.

Figure 57 shows the same filament photographed five minutes later, and the appearance of the nuclei in this figure is essentially the same as that shown in figure 56, except that the strand-like elements may be slightly darker and denser than in the preceding figure. Text-figure 6 shows the corresponding sector of



TEXT-FIG. 5. Diagram of a portion of the uppermost nucleus of FIG. 56, PLATE 12. FIG. 6. Diagram of a portion of the uppermost nucleus of FIG. 57.

the uppermost nucleus that was diagrammed in the previous figures. It is to be particularly noted in figures 56 and 57 that the nuclei are smaller than in the preceding figures. I have not observed swelling of the nuclei in water mounts, such as Schaeede (68, *pl. 3*) describes for the root tip cells of *Allium Cepa*. In most cases of antheridial cells of *Nitella* and *Chara* which I have kept under observation, the nuclei appear to shrink in size rather than to swell. This is further shown in figures 60, 61, 66, 67, 70, 71, 79, and 80.

In figures 56 and 57, there is a marked change in the appearance of the cytoplasm as contrasted with its more or less optical homogeneity in figures 52, 53, 54 and 55. Dark and dense irregular strand-like elements differentiated from a hyaline

ground mass now appear in the cytoplasm. This change parallels that occurring earlier in the nuclei.

A comparison of figure 57 with figure 52 suggests that the visible modification of the nuclei as a result of being mounted in water consists in a change from a more granular to a strand-like appearance of the denser nuclear elements. If we begin with the stage first visible, as shown in figure 68, the suggestion that a more homogeneous sol has changed to a gel with the withdrawal of a large amount of water from the dispersed phase is to be considered. The appearance of strands and irregular masses in figure 57 suggests that water may have been withdrawn from the interstices of protein colloidal particles, bringing them into closer proximity as series of granules and finally strands.

In figures 58 to 60 is shown another series of figures of a filament mounted in tap water. The terminal cell of this filament is obscured by extraneous material, which appears as the two conspicuous diagonal bars in figure 58. The lower cell is also obscured. The middle cell is well shown throughout the series, and in this case it is obvious that we cannot identify in the successive figures any particular nuclear elements such as could be recognized in the previous series (FIGS. 52-57). No definite granules are to be seen in these figures, either arranged in rows or groups. The dark elements in figure 58, photographed fifteen minutes after mounting, are perhaps best described as flocculated masses more or less connected, but not obviously reticulated. It is not clear whether the differences in the two series are due to differences in the stage of nuclear development or differences in the effect of the medium. Figure 59 shows the same filament five minutes later. The particular elements visible in the central nucleus in figure 58 cannot be so easily recognized as such in figure 59, though a diagonal line of four bodies toward the top of the nucleus is perhaps recognizable in both figures.

The same filament is shown thirty minutes after mounting in figure 60. Similarly in this figure, no particular nuclear elements present in the previous figures are readily identifiable as in the preceding series at this stage. In figure 60 the central nucleus has a shrunken appearance, and the cytoplasm shows irregular, clumped masses. At the points marked 1 and 2, are



large clear spaces that obviously suggest vacuoles. In figure 61, taken thirty-five minutes after mounting, the nuclei and the filament in general show a tendency to collapse.

The effect of once-distilled water on the appearance of the nucleus and cytoplasm is the same as that of tap water. Figures 62 to 67 show a further series of photographs of a filament mounted in once-distilled water made at intervals of five minutes. The nucleus marked *a* is perhaps typical for the series. Although the nucleus is very faintly differentiated in figure 62, the nuclear boundary is fairly sharp. The nuclear content at this time is still quite homogeneous, but with some suggestion of granules. However, the granules are by no means as clearly visible as in the first photograph (FIG. 52) of the tap water series. We can also recognize some dense, short strand or shred-like elements lying in the hyaline ground substance. There is already without doubt the suggestion in this figure of microscopically visible colloidal elements, granules, strands, and flocculated masses dispersed in a more hyaline continuum. Figure 63 shows the same nucleus five minutes later. As in the case of figures 52 and 53 we can identify in this figure particular light and dark elements that are recognizable in the preceding figure. These particular elements, very faint in figure 62, appear denser and darker and somewhat sharper in outline in figure 63. In figure 64, on the other hand, a marked change in the appearance of the nucleus is visible, and none of the particular elements that are present in figures 62 and 63 can be recognized in this figure. This is, at least, in part a matter of the photographing. The whole filament is darker and denser in appearance. Granules and strands are recognizable in the nucleus *a* as in the preceding figures, and these elements appear denser and larger. This increase in size and density of the dark elements suggests that the degree of dispersion of the discrete elements has perhaps decreased.

Figure 65, taken five minutes later, is strikingly like figure 64, and we can identify many corresponding elements in both figures. Figure 66, photographed thirty minutes after mounting, shows the same change noted at a corresponding stage in the preceding series. The nuclear elements consist of large, irregular, dense strands and flocculated masses that are not recognizable in figure 65. Moreover, the nucleus appears to have decreased

considerably in volume. The cytoplasm also shows dense clumps and flocculated masses, and does not have the same optical homogeneity that characterizes the previous figures.

In figure 67, taken thirty-five minutes after mounting, the changes noted in figure 66 are perhaps still more intensified. No particular strands, granules or flocculated masses that are present in the preceding figures are identifiable. The nucleus *a* is smaller than in figure 66. This series suggests that the effect of distilled water on the appearance of the nucleus and cytoplasm is very similar to that of tap water.

Figures 68 to 71 show a series of photographs made at different intervals of an antheridial filament mounted in one and one-half per cent cane sugar solution, made up with once-distilled water. Figure 68, photographed five minutes after mounting, comes the nearest to representing the appearance of a typical so-called resting nucleus when first mounted and before it has become affected in any way by the mounting medium. The nuclei marked *a* and *b* may be taken as typical of the filament. In figure 68, in contrast to figures 52 and 62 of the tap and distilled water series, very little except a faint nuclear boundary is to be seen, and, as noted before, the nucleus as a whole appears comparatively homogeneous. Figure 69, photographed thirty minutes after mounting, shows the differentiated elements recognized in the previous series. The nuclear boundary is sharp, and the nuclei have perhaps a more uniformly coarse granular appearance. The general appearance of the nuclei suggests that the hyaline areas represent a continuum in which the darker elements are dispersed.

In figure 70, the same filament photographed one hour after mounting, we see even more conspicuously than in the previous series the effect of long immersion in the sugar solution. The coarsely granular appearance of the nuclei has apparently disappeared, and in its place we find dense coarse strands and flocculated masses with irregular light areas between. The appearance of the cytoplasm has likewise undergone a decided change, with the appearance of strands and flocculated masses. The nuclei marked *a* and *b* in this figure do not occupy the central position in the cell as in figures 68 and 69, but appear to lie displaced against the cell wall. This condition is even more conspicuous in figure 71 of the same filament, taken two hours

after mounting. The nuclei marked *a* and *b* in this figure, as well as in figure 70, are much smaller than in figures 68 and 69, and appear somewhat shrunken. In one corner of each of these two cells we see large light areas that suggest vacuoles. Moreover, the nuclear boundaries in figures 70 and 71 are not as sharp as in the preceding figures, and there appears to be a layer of flocculated cytoplasm adhering to the nuclei.

The effect of a three per cent cane sugar solution on the appearance of the nucleus and cytoplasm is shown in figures 72 and 76. The successive changes in appearance in this series are much the same as in the water and one and one-half per cent cane sugar solutions, with the exception that apparently the changes take place more slowly than in the other media. As in figure 68 of the previous series, very little except a faint nuclear boundary is to be seen in the apical cell of figure 72, photographed five minutes after mounting, but in figure 76, two hours later, its nucleus has much the same appearance as the nuclei in figures 54, 55, 65, and 66. Filaments mounted in three per cent cane sugar solutions usually begin to swell after three or four hours, but swelling is not yet visible in figure 76.

Figures 77 to 80 show a series of photographs of a filament mounted in a five per cent cane sugar solution. Figure 77 shows four cells photographed five minutes after mounting, and in figure 80 we see the same cells six hours later. The changes in the appearance of the nuclei are of the same type as shown in the previous series. In figure 80 the cells are shown after six hours in the sugar solution, three times as long as was the case for the filament shown in figure 76. It is obvious in this figure that plasmolysis has occurred. In the regions marked *a* and *b*, the cytoplasm has obviously drawn away from the cell wall.

Figures 81 and 82, 83 and 84 show the effect of aceto-carmine, following a certain period in cane sugar solution, on the appearance of the nuclei. Figure 81 shows four terminal cells of a filament photographed five minutes after mounting in a three per cent cane sugar solution. The large conspicuous light and dark spots are due to extraneous material and are to be disregarded. The nuclear boundary in the second cell from the apex of the filament is sharp and clear, and the nucleus as a whole has a granular appearance with some suggestion of short strands. However, the light and dark elements are not clearly

differentiated, and, as in figure 68, the nucleus is comparatively homogeneous. The same nucleus is shown in figure 82 after being mounted five minutes in aceto-carmine. Ten minutes elapsed in making the change from the sugar solution to aceto-carmine. Considering the rapidity with which the appearance of the nucleus changes in sugar solutions, as is shown in figures 52 to 80, it is probable that the change in the nuclei shown in figure 82 may be due in part to the effect of the sugar solution. Nevertheless, the change is very marked. In figure 81, the second nucleus from the apex of the filament has a somewhat granular appearance; in figure 82, it shows many dense irregular fragments, short strands, and flocculated masses of material. None of these dense elements can be seen in figure 81, and they are apparently due to the action of the sugar solution and aceto-carmine. The nuclei in figure 82 are very similar in appearance to those in figures 54 and 55. A comparison of the second nucleus from the apex of the filament as it appears in figures 81 and 82 gives the impression that the aceto-carmine has brought about an aggregation of the denser elements into larger masses or aggregations, leaving conspicuous light areas between them.

Figure 83 shows three cells of another filament photographed after ten minutes in a three per cent cane sugar solution. Unlike the second nucleus from the apex in figure 81, the central nucleus in this figure already shows conspicuously differentiated elements. The nuclear boundary is very sharp, and within it the nuclear material gives more or less the suggestion of a reticulum. The filament shown in figure 83 was in the sugar solution five minutes longer before photographing than that of figure 81, and the difference in appearance between the two may in part at least be due to the effect of the longer exposure to the sugar solution. Figure 84 shows the same filament photographed five minutes later after being mounted in aceto-carmine. The effect of the aceto-carmine on the appearance of the nucleus is not as marked as in the case of figure 82, but is similar in its general character. It is to be noted particularly in figure 84 that we can recognize several of the same particular light and dark elements in the nucleus that are shown in figure 83. It appears from figure 84 that the aceto-carmine has not notably altered the appearance of the light and dark elements, but I regard figure 82 as showing

more typically the effect of aceto-carmine on the appearance of the nucleus.

In figures 85 to 90 is shown a series of photographs of four terminal cells of an antheridial filament mounted in aceto-carmine, made at intervals of several hours over a period of thirty hours. Figure 85 is a photograph made five minutes after mounting; figure 89 shows the same cells twelve hours later. Allowing for slight changes in appearance that may be the result of difference in length of exposure, development and printing of the negatives, these two figures are essentially alike as far as the appearance of the nuclei and cytoplasm is concerned. In figure 87, photographed three hours after mounting, there is no apparent change in the appearance of the nuclei, but the bulging of the cells between the cross walls suggests that they have begun to swell. This bulging becomes more marked in figure 88, photographed six hours after mounting. Figure 90 shows the four cells thirty hours after mounting. In this figure the outlines of the nuclear elements are not so sharp and clear as in the previous figures, and the filament as a whole appears much darker. This change is characteristic in aceto-carmine mounts kept for a considerable length of time. After twelve to eighteen hours the cytoplasm becomes rather diffusely stained with the carmine. The bulging of the cells, conspicuous in figures 87, 88 and 89, has apparently disappeared in figure 90. However, it is common in the aceto-carmine mounts for the cells to continue to swell until they burst.

Careful observation of preparations such as are shown in figures 81 and 82, 83 and 84, and 85 to 90, indicate that the changes that may result from treatment with aceto-carmine take place very quickly and that after these initial changes there is little essential modification in the appearance of the nuclei for several hours after mounting. In general the visible effect of aceto-carmine, made up according to Belling's formula and diluted in the manner previously described, on the appearance of the so-called resting nucleus in the antheridial filaments of *Nitella* and *Chara*, is to bring out the nuclear boundary more clearly and to cause what appears to be an aggregation of the denser nuclear elements visible in unstained and untreated nuclei into larger and more clearly differentiated irregular dark shreds, bodies, and strand-like aggregations.

The nuclear elements in such preparations as are shown in PLATE 12 are of course difficult to study and interpret, but in view of the many uncertainties as to the nature and behavior of the so-called chromatin granules, nucleole, nuclear sap, etc., it is certainly worth while to give renewed attention to their appearance in the living condition or as slightly modified by fixation as possible. These photographs illustrate the weaknesses of microphotography for showing cytological data, but they present at least an objective view of the mass changes that result from treatment of living cells with water, sugar and aceto-carmine solutions.

#### NUCLEAR AND CELL DIVISION IN ACETO-CARMINE PREPARATIONS

Figure 91 shows an aceto-carmine preparation photographed one hour after mounting, in which the nuclei stand out more conspicuously against the cytoplasm and appear more dense and opaque than in figure 68. This difference is probably due to the effect of aceto-carmine, as is shown in figures 81 and 82. Within the nuclei of figure 91 may be seen light and dark elements as in the figures in PLATE 12. In none of these nuclei in figure 91 can be seen the compound nucleole-like mass of material that is so conspicuous in the fixed and stained preparations shown in PLATE 12.

I have arranged as a series in figures 92 to 100, photographs showing the early prophase changes of the nucleus in aceto-carmine preparations. The seriation offers very great difficulties and the arrangement of the figures is merely tentative. The later stages of division following the equatorial plate are, of course, much more easily recognizable and their seriation is an easy matter, as Strasburger (74, 75) early showed in his studies on stamen hair cells of *Tradescantia*. I am presenting what I consider early prophase stages merely for comparison with the later stages after the chromosomes are fully differentiated, and regard the seriation presented as preliminary and requiring further study.

Figure 92 is from a photograph of a filament taken half an hour after mounting. The nuclei in this preparation appear less dense than in figure 91, but it is not certain that the stage shown here may not precede the stage shown in figure 91. In figure 93, photographed one hour after mounting, there is

perhaps the suggestion of a coarse reticulum. The light material between the dark elements also seems very clearly to be a continuum in which the dark elements lie. The appearance of strands and perhaps a reticulum is seen also in the apical nucleus of figure 94, photographed thirty minutes after mounting. The strands that apparently make up the reticulum appear comparatively thick and dense in certain regions and very faint and thin in other portions. This difference, however, may be due to certain portions of the reticulum lying out of focus.

What is perhaps a later stage than the one shown in figure 93 is seen in figure 95, photographed forty-five minutes after mounting. However, the suggestion of a reticulum of dark masses connected by thin, lighter strands is not as obvious as in the two preceding figures. The dense material is more uniformly dark, and the light material here also appears to form a continuum.

Figure 96, photographed two hours after mounting, certainly shows a much later stage than those shown in the preceding figures. The dense black elements in the nucleus of the terminal cell are considerably smaller in number but much larger in size than those shown earlier. There are approximately eleven large, intensely black, variously shaped bodies to be seen in this nucleus. Near the center of the nucleus are three large dense bodies, angular and rounded in outline and connected by thin strands of almost equal density. In the right hand portion of the nucleus is a conspicuous element with two hyaline spots in it, and there are other similar dense elements with pale areas in them. It is possible that in some cases these light areas may be high light spots of globular, dark elements. The dark bodies are visibly connected by longer or shorter thin dense strands. Similarly the hyaline areas between the dark elements are larger in size but comparatively smaller in number than in any of the preceding figures. The jagged and angular contour of the dark elements, so evident in figure 95, has disappeared to some degree in figure 96, and although still somewhat angular, these elements appear more rounded and elongated.

Figure 97 shows the apical cell of a filament photographed an hour and a half after mounting, in whose nucleus the suggestion of thick bands of dense material is very obvious. The dense elements in this nucleus and in the following stages are

sufficiently definite and small in number to be counted with some degree of certainty, and they, doubtless, are the chromosomes of fixed and stained preparations. There are approximately nine dark, conspicuous elements, numbered consecutively, in this nucleus. The presence of thin connecting strands between these bodies is not as evident as in the previous figure, and the dark elements are not as irregular and angular. Furthermore, in figure 97 the dense, thick band- or even ribbon-like elements give the impression of being somewhat curved and twisted. This is suggested particularly in the case of the elements numbered 1, 2 and 6. The appearance of the less dense material suggests that it is a part of the bands lying somewhat below the focal plane.

In figure 98, taken one hour after mounting, the short bands or ribbon-like elements appear sharper in outline than in the previous figure, and the hyaline areas between are likewise more clear and sharp. We can count in this photograph approximately seven dense, black elements which still appear somewhat connected. Element number 2 is obviously connected by a thin dense strand with number 1, and similarly the elements numbered 3, 4 and 5 appear connected. The hyaline area numbered 9 has the shape of a wide U, and extends downward between elements 7 and 2, forming a light zone that resembles a distorted Y. Number 8 is another long hyaline area extending from element number 5 to the thin connecting strand between elements 1 and 2. No nuclear boundary is visible around the upper part of the nucleus, but around the lower half may be seen a fine line which suggests the so-called nuclear membrane.

Figure 99, photographed two hours after mounting, is perhaps a later stage than that shown in the previous figure. In the lower right hand portion of this nucleus may be seen a thick dense band that extends half way around the periphery of the nucleus from the point marked 4 to 9 on the other side. In the center of the nucleus lies another band, numbered 3, that appears somewhat coiled and twisted, and seems to disappear below the focal plane at 1. At 5 is a straight band somewhat club-shaped at the upper end, and the element marked 8 and 6 appears to be a single mass of material whose central portion perhaps lies below the focal plane. The dense rounded element at 7 is connected with element number 3 by a thin dense strand.



The suggestion is very obvious in this nucleus that the hyaline zones constitute an optically structureless continuum in which the band- and ribbon-like elements lie.

In the central cell of figure 100, photographed one hour after mounting, we get the impression of a somewhat rounded group, with a tendency for the elements to be parallel and concentric. Extending from the region marked 1 to 9, the elements appear somewhat continuous. In the region labeled 2 is a curved, rather pale band which connects with the same band at 3. At 4 and 10, we get the suggestion of strands which are rather pale. The dense element marked 6 appears to be a short portion of a ribbon, and at 7 and 8, we have two other dense irregular bands. Figure 100 is more aberrant in its more generally hyaline and homogeneous appearance. Figures 91 to 100 show certain changes which occur in the prophase stages of the nucleus in antheridial filament cells of *Nitella* and *Chara*. Their interpretation is difficult and the seriation given, as noted, is tentative.

Figures 101 to 103 may represent the same stages as seen in slightly higher magnification. In the central nucleus of figure 101, I have numbered consecutively three thick bands extending diagonally across the center of the cell. At 5 is a short thick element. In figure 102 the nuclear boundary seems to be still present. In this cell, photographed two hours after mounting, the dense elements still appear more or less connected. At the upper pole of the nucleus is a conspicuous band, marked 7, and extending from 1 to 5 is an interrupted element, parts of which seem to lie below the focal plane and give the impression of being curved and twisted. At the points marked 1, 2 and 5, it is very dense. Across the center of the nucleus is a thick dense mass of material, which appears to extend with varying thickness and density from 9 to 6. At 4 it appears very dense and thick, and at its lower end it appears to be connected with the element labeled 3. At 8 and 3 are two other dense bands. In figure 103, two large chromosome-like elements are conspicuous at 5 and 6, but the other dense elements numbered consecutively in this nucleus are not as sharp and distinctly separated as these. In this nucleus we still get the suggestion of a bounding membrane around the nuclear elements.

If we compare the nuclei in the successive figures from 91 to 103, we are impressed with the apparent increase in size but

decrease in number of the dense elements as the prophase changes progress, until in figure 97 we can begin to count them. In figures 99 to 103, a relatively small number of very large, dense elements appear lying in a more or less optically homogeneous ground substance.

I have not seen in living cells and aceto-carmin preparations definite spindle fibers such as are present in fixed and stained cells, except perhaps in figures 107 and 108. No polar caps or felted zones are recognizable in any of my preparations, nor could distinct fibers be seen in equatorial plate, anaphase, and telophase stages. In a number of cells in late anaphase and telophase stages, however, I have seen faint thread-like elements in the cytoplasm between the chromosomes and daughter nuclei that suggest the presence of spindle fibers. In figures 107 and 108 we see evidence of spindle fibers. Figure 108 shows a late anaphase or early telophase of two cells in which distinct fiber-like and cell plate elements are visible between the daughter nuclei. This filament had been mounted in tap water twenty minutes previous to photographing, and I have not so far obtained further figures confirming the appearances shown in it. Figure 107, an aceto-carmin preparation, shows four terminal cells of a filament in which fiber-like elements are visible between the anaphase groups of chromosomes. However, the basal end of this filament had been injured. It is to be further noted that in none of the other aceto-carmin preparations shown in PLATE 13 are spindle fibers visible.

Strasburger (74, 75), Behrens (3), Lundström (38), Samassa (65), Zacharias (87), Fischer (21) and Sands (66) failed to find evident spindle fibers in living cells of *Tradescantia*. Demoor (12), De Wildeman (13) and Schaede (68), on the other hand, figure them in abundance in this genus. In *Spirogyra*, Strasburger (75) describes the appearance of spindle fibers in living cells. De Wildeman has also figured them for *Spirogyra*, but his observations are contradicted by Behrens. In a few cases in *Epipactis* and *Orchis*, Treub (81) describes spindle fibers in living cells, but Zacharias fails to report such structure in living cells of *Larix*, *Cucurbita* and *Helleborus*. The later investigations of Lundegårdh (37) on *Allium*, *Vicia* and *Cucurbita*, and of Chambers and Sands (8) on *Tradescantia* indicate that spindle fibers are not visible in living plant cells. Schaede (68, pp. 240-

241), however, in a later study than Lundegårdh's, maintains that spindle fibers are plainly visible in living cells of *Allium*, and Showalter (72, fig. 3) figures spindle fibers in living germinating spores of *Pellia*.

Figures 105 to 115 show stages which appear to correspond to the so-called equatorial plate, metaphase, anaphase, and telophase stages of fixed and stained preparations. Figure 105, photographed one hour after mounting, shows four cells, in one of which, *A*, the chromosomes are in the equatorial plate stage. The large size of the chromosomes of this species of *Nitella* is very evident in this cell. The long chromosome 1 extends from the equatorial region at 4 to almost the end of the cell at 1. The chromosomes marked 2 and 3, as well as the two labeled 1 in cell *B*, show a light central line throughout their length, but it is not obvious here whether this light median line is evidence of a longitudinal split or whether it is a high light effect of the refractive chromosomes when slightly out of focus. A somewhat similar light central line is visible in the late anaphase chromosome, marked 1, in figure 109.

Figure 106 shows two cells, photographed two hours after mounting, in which the chromosome groups are in a late anaphase or diaster stage. The chromosomes of *Chara sp.* are so numerous and close together that it is difficult to bring them out individually in photographs. In the terminal cell of this filament, however, we can see a thick, U-shaped chromosome, marked 1. It is to be noted that the daughter groups of chromosomes in the lower cell lie almost parallel to the long axis of the filament, while in the upper cell they are diagonally placed. It is obvious from such a figure that if the cross wall, which is to be later formed between the daughter nuclei, is to be transverse to the filament, the whole division figure will have to rotate approximately 90 degrees. A later stage is shown in figure 109, taken an hour after mounting, in which the chromosomes have reached the ends of the cells. Figures 109, 110, and possibly 111, resemble the diaster stages of fixed and stained preparations.

In figure 109 the outlines of a few of the chromosomes are visible, and although not individually differentiated in figure 110, the orientation of the groups of chromosomes as a whole suggests that of a diaster. The position of the groups of chromosomes in the cells of these figures is noteworthy. In figure 110, most

of the daughter groups of chromosomes lie in diagonally opposite corners of the cell. This is even more pronounced in figure 111. In nine cells of this filament the groups of chromosomes lie in diagonally opposite corners. Moreover, in the cells between 1 and 2, the long axis of the division figures in adjacent cells is not parallel, and lines drawn so as to pass through the long axes of all of these division figures would be more or less regularly zigzag.

Figure 111 appears to show a later stage than figure 110. In this stage the chromosomes are closely massed together, and it is impossible to identify them individually. In some of these cells the chromosome groups are triangular in shape, conforming to the corners of the cells in which they lie. This is particularly noticeable in the cells marked 2 and 3.

Figure 112 probably represents an early telophase. The appearance of the daughter nuclei in these two cells gives the impression that they are in the so-called dispireme stage of fixed and stained preparations. The nuclei are flattened in shape and lie in the ends of the cells, but it is not very clear whether nuclear boundaries are present. Near the center of the nucleus marked *x*, lies a dense dark body, labeled 1, which suggests a nucleole. However, it does not have the characteristic appearance of the nucleoles shown in figures 52 and 53. Around the periphery of the nucleus between the points marked 2 and 3, the dark elements appear continuous, but this may be due to overlapping. At the regions marked 3 and 5, this continuous dark element appears to be connected with the dense element labeled 4. There are large, conspicuous hyaline areas in this nucleus; one in the center in which the nucleole-like body lies, and one on each side of the central area. As in the prophase nuclei, these hyaline areas appear to constitute an optically homogeneous ground substance in which the dense elements lie.

What is perhaps a still later stage is shown in figure 113, photographed one hour after mounting. The nuclei in this figure are more uniformly dark, and the dense elements consist of irregular strands and angular bodies. In the terminal nucleus of this filament, however, we can still see some evidence of longer, irregular band-like elements. Four such strands are numbered consecutively in this nucleus. On the right of the nucleus is a long dense band, marked 1, and more or less parallel

to it are three other irregular strands, apparently connected at places by lighter, thin elements.

In figure 115, the nuclei have a more rounded outline, and the suggestion of long strands and dispireme threads has disappeared. However, in the regions of the apical nucleus of this filament, which I have indicated by numerals, we see suggestions of strands that are slightly denser and darker than the other elements in the nucleus. The general appearance of the nucleus is very similar to that of resting nuclei.

The changes that have apparently occurred in the appearance of the nuclei in the figures from 111 to 115 give the impression that the large, dense elements—chromosomes—of the late anaphase break up into smaller and smaller aggregates as the telophases progress, until a state is reached where the daughter nuclei have the appearance of resting nuclei. The dense elements appear to become more highly dispersed.

The process of cell plate formation is difficult to see in living cells and aceto-carmin preparations. As noted above, I have not seen definite spindle fibers in living and aceto-carmin treated material, except perhaps a few traces of such structures in late telophase stages. In figure 108, as previously noted, definite fiber-like elements are visible between the daughter nuclei, and across the equator of what look like spindles in these cells may be seen a faint lighter zone, whose appearance suggests an early cell plate. In figure 114, the cell plate appears as a faint line between the telophase nuclei, and in figure 115, it is fully formed and in contact with the horizontal walls of the cell.

The observations presented from fixed and stained preparations, together with the appearances shown in these aceto-carmin preparations, are strong evidence that cytokinesis in *Nitella* and *Chara* takes place by the progressive growth and development of a definite cell plate, which is later followed by a cell wall in the same manner as in the higher plants.

#### DISCUSSION

The changes occurring in the appearance of the nuclei and cytoplasm of antheridial filament cells of *Nitella* and *Chara* when mounted in water and sugar solutions are fairly definite and constant. When so treated, the outlines of the nuclei be-

come sharper and more distinct and their contents coarser, more granular and reticulum-like. Nägeli (51, *fig. 11b*) reported an injurious effect of water on the antheridial filament cells of *Nitella flexilis* as early as 1844, and both Nägeli (50) and Von Mohl (47) described contraction of the cellular content of pollen and spore mother cells as a result of being mounted in water. A few years later, Hofmeister (28, p. 428; 29, *fig. 2*, p. 6) found that pollen mother cells became quickly disorganized after being mounted in water. In some instances, he reports that so-called coagulation took place before the cells could be brought into focus. In 1880, Baranetzky (1) and Strasburger (74, p. 146-147; also 76, p. 299) report an increased clearness and sharpness in outline of the nuclear elements as a result of their being mounted in water. Lundegårdh does not specifically recognize any change in appearance of the nucleus and cytoplasm of root tip cells of *Allium Cepa* and *Vicia Faba* as a result of treatment with water and sugar solutions, but he reports that the structure of the nucleus is better differentiated in a preparation one-half to one hour old than in one freshly mounted.

These changes in the appearance of the nucleus as a result of being mounted in water and sugar solutions, as shown in PLATE 12 of my preparations, suggest possibly a closer and closer approach of colloidal particles or aggregates by withdrawal of water from the dispersed phase, until microscopically visible, irregular granules, bodies, and strands, such as are shown in figures 56, 57, 59, 60, 61, 66, 67, 70, 71, 79 and 80 are formed. The gradual emergence of conspicuous vacuoles in the cells as the appearance of the nucleus changes is perhaps the visible expression of similar changes in the cytoplasm.

As is shown in figure 68 of my preparations, and by Schaeede (68, *pl. 3, figs. 1a, 3, 4*), differentiated nuclear elements are not microscopically visible in the cells when they are first mounted. This is not, however, adequate ground, it seems to me, for assuming that the resting nucleus is not colloidal. The indices of refraction of the different phases may be so much the same that little structural differentiation is visible. On the other hand, it is possible that the hydrolytic dispersion of the dispersed phase is so great that no microscopically visible elements are present when the cells are first mounted. It is none the less obvious in the different series shown in PLATE 12 that the apparent decrease in

the degree of dispersion of the denser elements as the changes continue is paralleled by an apparent increase in their size. These changes, it seems to me, appear to be similar in many respects to the changes that occur in the aging of dilute starch, described by Ostwald and Fischer (56). According to these authors, there occurs in the aging of dilute starch not only a decrease in dispersion but also a dehydration of the dispersed phase. The colloid particles not only appear to give off a part of their water, but they clump together to form larger aggregates. In the figures shown in PLATE 12, it appears as if the water that is perhaps withdrawn from the interstices of the dispersed colloidal protein phase accumulates in certain regions of the cell, forming large vacuoles, such as are shown in the cells marked 1 and 2 in figure 60, and *a* and *b* in figures 70 and 71.

The assumption that these changes in the appearance of the nucleus and cytoplasm are indicative of injury or death, and that the dispersed protein particles have aggregated together into microscopically visible particles by withdrawal of water from their interstices, is in line with the well known fact that protoplasm which has been injured in any manner, by frost, for example, as described by Nägeli (52) and Molisch (48), or by heat and chemical agents, is more permeable. It allows the cell sap, which in living and growing cells is always subject to high pressure, to diffuse out as if it had become porous. This is well shown by the familiar fact that when cells with coloured sap are frozen or heated to high temperatures, the pigment diffuses out readily, though this does not occur as long as the cells are living. Pfeffer's (59) extensive observations, duplicated in part by later studies by Fischel (20), Overton (58), Ruhland (64), Meyer (42) and Schaede (67), on the absorption of aniline dyes by protoplasm, indicate further an increased permeability of the cell after death. Pfeffer found that living protoplasm is impermeable to aniline dyes, and only after it is injured or dead are these stains permitted to penetrate the plasma membrane and other parts of the cell.

That the earlier changes in appearance of the cells shown in PLATE 12 are indicative of death or the approach of death, is not to be regarded as proved. They are, none the less, very similar to the so-called disorganization appearances accompanying death described by Strasburger (74), Klemm (35) and Schaede

(68). Strasburger reports that when pollen mother cells of *Tradescantia* are first mounted in well water, nothing is to be seen in the nuclei, but very quickly as the water begins to affect the cells, the nuclear elements become coarser and more clearly differentiated. This change continues, as death approaches, until the nucleus and cytoplasm are completely disorganized. Klemm found that treatment of the protoplasm of various plants with intense light, high and low temperatures, acids, alkalis, and other substances, produced marked changes in appearance as the cells died. The disorganization appearances, as he reports them, were extremely varied and differed according to the rate of death, whether slow or rapid. In general, Klemm describes the changes as granulation, vacuolization, coagulation and precipitation. He reports that as the protoplasm dies, it may take on a fibrillar appearance or become granular or vacuolated; the granules often uniting to form chains and net-like structures. Although Hofmeister (29) claims that contraction is a general and consistent accompaniment of death, Klemm found that this is not universal, even when there is an immediate loss of turgor. He reports the changes in the appearance of the nucleus to be the same as in the cytoplasm. In the root tip cells of *Allium Cepa* and *Vicia Faba*, Schaede (68) finds that the nucleus is optically homogeneous when first mounted in water and sugar solutions, but after the elapse of one-half to one hour, it becomes swollen and its content coarsely granular. He interprets this change in the nucleus as indicative of the gradual death of the cell. Similar modifications in the appearance of the nucleus are reported by Miehe (43, fig. 8) and Nestler (54) for cells in the vicinity of other mechanically injured cells.

Pfeffer (60) regards absorption of dyes, non-plasmolyzability, and permeability of the protoplasm to soluble pigments in the cell sap as indicative of death. Bechold (2, p. 302) states that with the occurrence of death, protoplasm gelatinizes and appears ultramicroscopically as a conglomeration of reflecting platelets. If the protoplasm is killed quickly, stiffening occurs; if it dies slowly, flocculation takes place. It is to be noted in connection with Bechold's observations, that in figures 58, 59 and 60 of my preparations, the central nucleus has a highly flocculated appearance. Chambers (7) notes that in the death changes the cytoplasm "may suddenly set, forming a rigid coagulated



mass. The coagulation structure gradually coarsens with the production of a network or granular precipitate."

It is to be particularly noted in the series of figures shown in PLATE 12 that the nuclei are apparently the first parts of the cell to change in optical appearance. Schaede (68) likewise emphasizes this for the root tip cells of *Allium* and *Vicia*. It is only after the elapse of several minutes that a marked change is visible in the appearance of the cytoplasm. It was particularly noted by Klemm (35) and Pfeffer (60) that the different parts of the cell do not all have the same powers of resistance to poisons. Pfeffer (59) found that the nucleus would often take up a stain while the cytoplasm remained entirely unstained; and it often happened that a poison killed the nucleus before the cytoplasm was fatally injured. Klemm reports that the nucleus is poor in resistance to electric shock, and that the nucleus can be killed while the cytoplasm remains alive for several hours. In a similar manner, it may be that the tap and distilled water change the appearance of the nucleus before the cytoplasm is affected.

The change in position, shape and appearance of the nuclei as a result of mechanical injury to the antheridial filaments is noteworthy. As previously described for figure 51, not only is the appearance of the nuclei in the immediate vicinity of the injury affected, but in less degree that of nuclei far removed. As noted before, I have observed the effects of mechanical injury on the position, shape and appearance of nuclei thirty cells removed from an injured cell. According to the observations of Tangl, Nestler, Miehe, Schürhoff, and Němec, this wound reaction of the protoplasm of neighboring cells is general in the plant kingdom. The reaction may even involve the passage of the nuclei through the cell wall nearest the injury (Miehe 43, *figs. 2, 3, 4*). Nestler reports that the effect of mechanical injury to a cell in the epidermis of *Tradescantia viridis* could be detected by changes in the position and appearance of the nuclei within a radius of .7 to .8 mm. of the injury. Miehe finds the maximum distance to which such reactions may extend is often as much as 1.8 mm. Figures 1 and 8 by Nestler show, in every cell in the vicinity of the injury, the nuclei lying in a dense mass of cytoplasm against or in close proximity to the cell wall nearest the injury. These figures suggest a general

flow of the protoplasm in the direction of the injury. Not only does the position of the nuclei change, but their appearance also, as is shown by Miehe's figures 8a and 8b, and figure 51 of my preparations. Miehe describes the nuclei of the cells lying near the injury as being very coarse and granular in appearance. In his figures 5, 6 and 7, each nucleus appears much denser and more granular on the side toward the injury, as if a stream had passed through the nucleus in the direction of the injury, carrying the granules and particles in the nucleus with it.

It is to be noted, however, that the observations of the above investigators, with the exception of Miehe, were made several hours and even days after the injuries were made, and they do not record any immediate changes such as are shown in figure 51 of my preparations. Miehe, on the other hand, describes marked changes in the cells and nuclei thirty minutes after injury. In the light of these observations, it seems questionable whether any living tissue, such as the root tips of *Allium* and *Vicia* used by Lundegårdh (37) and Schaede (68), which has first to be sectioned, is suitable for study of nuclear and cell division in living material. It is questionable whether any living root tip can be sectioned thin enough for study under the high powers of the microscope by the methods commonly in practice and still not show injury effects in the nuclei and cytoplasm. Although Lundegårdh and Schaede undoubtedly selected cells whose nuclei and cytoplasm showed no signs of injury, it is not improbable, in the light of Miehe's and Nestler's observations, that microscopically invisible changes had been initiated as a result of the sectioning, which later affected the appearance of the nucleus and cytoplasm.

The photographs shown in PLATE 13, as noted before, are representative of the changes in the nuclei in the antheridial filament cells of *Nitella* and *Chara* as they divide. If we compare the very earliest prophase changes with the changes that occur in the appearance of the nuclei as a result of being mounted in water and sugar solutions, as described for PLATE 12, we are impressed with a certain, and perhaps misleading similarity of the two processes. In the different series shown in PLATE 12, the dense elements in the resting nuclei apparently become less dispersed and more aggregated until large granules, bodies and strands are visible; and similarly in the earliest prophases there

is perhaps a decrease in the dispersion of the dense elements, accompanied by a tendency to aggregation and increased size of the dispersed elements. However, much further study is needed to show the real nature of these changes and their relation to the later stages of chromosome formation.

The presence of an irregular, coarsely granular reticulum with more or less uniform nucleoles in the resting nuclei of aceto-carmin and unfixed and unstained preparations of *Nitella* and *Chara* is strong evidence against the origin of the spireme and chromosomes from an aggregated nucleole-like mass of material. While the highly stainable, aggregated body which is so often present in resting nuclei of fixed and stained preparations frequently decreases in size and appears to lose some of its contents in the prophase, I find no adequate evidence that its material goes to make the spireme and chromosomes. In the late prophase, as shown in figures 10 and 12, the aggregated mass has apparently disappeared, and a more or less rounded, single nucleole is present. In figure 9, on the other hand, a deeply stained aggregated mass of material is still present, although the spireme segments are fully formed. It is difficult to differentiate a true nucleole in the aggregated mass seen in fixed preparations, although definite rounded bodies are shown in figures 2, 3, 29, and 31. The staining reaction of the mass as a whole is generally the same throughout, but a number of preparations have been found (FIGS. 29, 30, 31) in which a large body has stained intensely red in contrast to the violet color of the surrounding fragments. In the early prophase shown in figures 34 and 35, the staining reaction of the granules and fragments is quite different from that of the round, nucleole-like bodies. In Flemming's triple stain, the granules, fragments and strands are a deep violet in contrast to the ruby red of the rounded nucleole-like bodies.

In the telophases the chromosomes are transformed into typical dispirome threads, whose staining reaction is distinct from that of the rounded bodies which begin to appear in the daughter nuclei at this stage. While the evidence from fixed and stained material is not at all complete, the conditions found in unfixed and unstained and aceto-carmin preparations of resting and prophase nuclei point to the origin of the spireme and chromosomes in *Nitella* and *Chara* from an irregular and somewhat granular reticulum.

The view of Zimmermann, Kaiser, Debski, and Riker, that the numerous deeply stained irregular and rounded bodies in the cytoplasm of *Nitella* and *Chara* are extra-nuclear material or bodies extruded from the nucleus, and particularly formed by the disintegration of the nucleole during division, seems improbable in the light of the conditions shown in figures 19, 23, 25, 27, and 28. It is difficult to explain the large masses of bodies or granules here shown as coming from the nucleus, and particularly the nucleole, during mitosis, unless, of course, they are assumed to grow and divide when they get into the cytoplasm. The volume of the granules lying in the cytoplasm of figures 19 and 23 appears to be almost equal to that of the chromatin of the entire nucleus, and the mass of highly stainable material present at the poles of the spindle shown in figure 28 is equal in volume to the whole group of chromosomes in the equatorial region. According to the view that these bodies are extra-nuclear nucleoles extruded from the nucleus during mitosis, we would naturally expect to find them more numerous during division stages than in resting cells, unless we assume that they persist from one cell generation to another. Yet in figures 23, 24, and 25 of resting cells, fully fifty deeply stained bodies of various sizes and shapes are present, while in the dividing cells shown in figures 11, 12, 13, 16a, 17, 20 and 21, comparatively few such bodies are visible. The contrast between the number of bodies in resting and dividing cells is not always as marked and general as noted in the cells just referred to, but such cases indicate that highly stained chromatic bodies may occur in the cytoplasm regardless of whether the nucleus is undergoing division or not. The appearance of the bodies in the equatorial region of the spindle shown in figure 27 is somewhat like that of nucleoli, but their large size and number again render it highly improbable that they are of nucleolar origin. The fact that these granules are not destroyed by fixatives containing acetic acid, coupled with their difference in size and staining reaction from chondriosomes as commonly shown, indicates that they are of a different nature from these latter bodies.

While it is true that small bodies or granules frequently occur near or in contact with the nuclear membrane during prophase and at the poles of the spindles in metaphase and anaphase,

they are not, it seems to me, to be regarded as centrosomes, as held by Schottländer and Kaiser. As previously noted in figure 1, a number of granules lie near the nuclear membrane, and two of them appear somewhat like two large centrosomes that have resulted from a previous division. In figure 11, two small and two relatively large bodies lie at the opposite sides of the nucleus, while the two bodies lying at the poles of the spindle shown in figure 17 are very irregular in shape and as large in size as some of the small chromosomes in the equatorial region. A comparison of the granules and bodies in these figures with those shown in figures 16a, 16b, 18, and 19, shows little difference in size, shape and staining capacity between the two. It seems evident from a comparison of the preparations that the bodies frequently occurring near or in contact with the nuclear membrane and at the poles of the spindle are of the same type as the bodies lying variously scattered in the cytoplasm, and differ from the latter only in size and position in the cell. No astral rays have been seen in connection with these bodies, and the spindle appears to be formed independently of their presence. Some evidence of radial kinoplasm may be seen in figure 11, and faint indications of polar caps are present in the cell of an antheridial filament shown in figure 40; but I have not been able to determine with certainty the mode of origin of the spindle in *Nitella* and *Chara*.

The difficulty of using photographic methods on cytological material, as noted before, particularly living cells and acetocarmine preparations, is evident in the figures shown in PLATES 12 and 13. The figures in PLATE 13 are inadequate for bringing out the cytological details of mitosis, but they show certain of the mass changes that occur in the colloidal protoplasm during division. It is becoming increasingly evident from the study of matter in the colloidal state that the so-called cell organs, nucleole, chromatin granules, linin, nuclear sap, etc., are elements in a complex colloidal system, and possibly in mitosis pass from one state to another. In the light of this evidence and the fact that our attention is becoming more and more centered on the mass changes in the cellular colloid system as it grows and divides, it is certainly worth while to give renewed attention to nuclear and cell division in living cells, and material as little modified by fixation as possible. The antheridial filaments of the Characeae, in my opinion, are specially

favorable material for such study. I am continuing my studies on this material and regard my results so far as preliminary. The possibility of checking the appearances obtained in fixed and stained material by the study of living cells and *intra-vitam* staining, it seems to me, is of great importance.

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### Explanation of plates 10-13

All figures were drawn with the aid of a Spencer camera lucida and a Zeiss 2 mm. apochromatic objective N. A. 1.30 and compensating oculars 6, 8, 12 and 18. The approximate magnifications are given with each figure. The photographs in plates 12 and 13 were made with the same objective and compensating oculars 6, 8 and 12, with a Leitz Liliput arc light as the source of illumination. The approximate magnification is given with each photograph.

### Plate 10

FIG. 1. Nodal cell of *Nitella gracilis* showing a mass of densely stained material at the center of the nucleus and numerous irregular granules in the cytoplasm.  $\times 1800$ .

FIGS. 2 and 3. Nuclei of mature eggs of *N. gracilis* showing similar masses of deeply stained material in the center. Vacuolated nucleoli lie close to the dense masses in the center.  $\times 2400$ .

FIG. 4. Nucleus of an apical cell of *N. gracilis* with four deeply stained, rounded bodies in the nuclear reticulum.  $\times 1800$ .

FIG. 5. Nucleus of an egg of *N. gracilis* showing chromatic reticulum as in FIG. 4, and knob-like projections of the aggregated mass at the center.  $\times 1250$ .

FIG. 6. Nucleus of a nodal cell of *N. gracilis* showing chromatic reticulum as in FIGS. 4 and 5, and seven densely stained bodies closely grouped together.  $\times 1800$ .

FIG. 7. Nucleus of an apical cell of *N. gracilis* with numerous dark bodies in the nuclear reticulum and knob-like projections from the central mass of material.  $\times 1800$ .

FIG. 8. Nucleus of an apical cell of *N. gracilis* drawn from an aceto-carmin preparation with a rounded nucleole and a granular reticulum.  $\times 1800$ .

FIG. 9. Late prophase nucleus of a young manubrium of *Chara sp.* showing irregular spireme segments together with a deeply stained, irregular mass of material.  $\times 2400$ .

FIG. 10. Late prophase nucleus of an apical cell of *Chara sp.* showing nodular spireme and a somewhat constricted nucleole lying next to the nuclear membrane.  $\times 3400$ .

FIG. 11. Late prophase stage in a cell of the 2-cell stage of an antheridium of *N. gracilis*. The nucleus is filled with an even, convoluted spireme, and the cytoplasm around the nucleus suggests a radial arrangement. Two large and two small granules lie at the opposite poles of the nucleus.  $\times 1800$ .

FIG. 12. Late prophase in a cell of the 4-cell stage of an antheridium of *N. gracilis* showing a round nucleole and approximately 32 spireme segments.  $\times 3400$ .

FIG. 13. Polar view of the first division of the nucleus in the 1-cell stage of an antheridium of *N. gracilis* showing 34 chromosomes and 7 chromatic bodies.  $\times 1250$ .

FIG. 14. Polar view of an equatorial plate of a nodal cell of *N. gracilis* showing 34 chromosomes.  $\times 1800$ .

FIG. 15. Polar view of late prophase or equatorial plate in an antheridial filament cell of *N. gracilis*.  $\times 1800$ .

FIGS. 16a and 16b. Early metaphase in a cell of the 8-cell stage of an antheridium of *N. gracilis*.  $\times 3400$ .

FIG. 17. Early anaphase in a cell of the 4-cell stage of an antheridium of *N. gracilis* showing numerous granules of various sizes in the cytoplasm and the spindle converging at the poles toward two large, deeply stained bodies.  $\times 2400$ .

FIG. 18. Late anaphase in a cell of the 4-cell stage of an antheridium of *N. gracilis* showing the broad, barrel-shaped spindle.  $\times 2400$ .

FIG. 19. Dispireme in a cell of the 4-cell stage of an antheridium of *N. gracilis*. The daughter nucleus at the upper end of the cell is almost obscured by large, deeply stained bodies in the cytoplasm. Numerous similar bodies are also present near the equator of the spindle.  $\times 2400$ .

### Plate 11

FIG. 20. Late telophase in a nodal cell of *N. gracilis* showing the thickening of the spindle fibers in the equatorial region. In the center of the daughter nuclei are large masses of deeply stained material.  $\times 2400$ .

FIG. 21. Early cell plate stage in a cell of the 4-cell stage of an antheridium of *N. gracilis*. The cell plate has not yet reached the walls of the cell.  $\times 1800$ .

FIG. 22. Late cell plate stage in a nodal cell of *N. gracilis*.  $\times 1800$ .

FIG. 23. One-cell stage of the antheridium of *N. gracilis* showing approximately fifty deeply stained granules and bodies of various shapes and sizes lying in a somewhat semi-circular group around the nucleus in the upper part of the cell, marked x.  $\times 1250$ .

FIG. 24. Tip cell of a young oogonium of *N. gracilis* after the first 'Wendungszelle' has been cut off, showing a mass of granules and bodies on the side of the nucleus towards the external surface of the cell. Practically no granules and bodies are present in the 'Wendungszelle'.  $\times 1250$ .

FIG. 25. Egg cell of *N. gracilis* with two 'Wendungszellen' showing approximately fifty deeply stained and variously shaped granules or bodies in the upper part of the egg cell. Very few bodies are present in the 'Wendungszellen'.  $\times 1800$ .

FIG. 26. Nodal cell of *N. gracilis* fixed in Benda's fluid and stained according to Benda's method for chondriosomes. The large bodies or granules are distinct from the chondriosomes.  $\times 1800$ .

FIG. 27. Late anaphase stage in a nodal cell of *Chara sp.* showing eleven large dense bodies in the equator of the spindle.  $\times 2400$ .

FIG. 28. Equatorial plate stage in an apical cell of *Chara sp.* showing dense masses of bodies of various sizes and shapes at the poles of the spindle. The bodies are more numerous at the upper pole of the spindle.  $\times 3400$ .

FIGS. 29-33. Nuclei from resting cells of the antheridial filaments of *N. gracilis* showing numerous deeply stained bodies in the nuclear reticulum.  $\times 2400$ .

FIGS. 34-36. Prophase changes in the nuclei of antheridial filaments of *N. gracilis*.  $\times 2400$ .

FIG. 37. Nucleus of an antheridial filament cell of *Chara sp.* with a nodular spireme.  $\times 2400$ .

FIG. 38. Late prophase nucleus of *Chara sp.* showing numerous spireme segments, three of which appear longitudinally split.  $\times 3400$ .

FIGS. 39 and 40. Late prophase nuclei in antheridial filament cells of *N. gracilis* showing some evidence of polar caps.  $\times 2400$ .

FIG. 41. Equatorial plate stage in an antheridial filament cell of *N. gracilis*. The chromosomes lie diagonally placed in the cell.  $\times 2400$ .

FIGS. 42 and 43. Polar views of equatorial plate stage in aceto-carmine preparations of antheridial filament cells of *Chara sp.* showing variation in size and shape of the chromosomes in different cells.  $\times 2800$ .

FIG. 44. Equatorial plate stage in the terminal cell of an antheridial filament of *Chara sp.* showing the equatorial plate parallel to the long axis of the filament.  $\times 2400$ .

FIG. 45. Early anaphase in a cell of an antheridial filament mounted in aceto-carmine. No spindle fibers are visible.  $\times 2800$ .

FIG. 46. Metaphase or early anaphase in an antheridial filament cell of *N. gracilis*.  $\times 2800$ .

FIG. 47. Late anaphase in an antheridial filament cell of *N. gracilis* with the division figure diagonally placed in the cell.  $\times 2400$ .

FIG. 48. Later anaphase in two antheridial filament cells of *Chara sp.* showing barrel-shaped spindle lying in a diagonal position in the cell.  $\times 2400$ .

FIG. 49. Early cell plate in two antheridial filament cells of *Chara sp.* The telophase nuclei appear flattened against the end walls of the cells.  $\times 2400$ .

FIG. 50. Late cell plate stage in an antheridial filament cell of *N. gracilis*. The cell plate is completely formed across the equator of the cell. Spindle fibers are still present, however.  $\times 2400$ .

### Plate 12

FIG. 51. Antheridial filament of *Chara sp.* showing change of shape, position and appearance of the nuclei as a result of mechanical injury.  $\times 1000$ .

FIGS. 52-57. Portion of an antheridial filament of *Chara sp.* mounted in tap water.  $\times 1000$ . FIG. 52. Ten minutes after mounting. FIG. 53. Fifteen minutes after mounting. FIG. 54. Twenty minutes after mounting. FIG. 55. Twenty-five minutes after mounting. FIG. 56. Thirty minutes after mounting. FIG. 57. Thirty-five minutes after mounting.

FIGS. 58-61. Portion of an antheridial filament of *Chara sp.* mounted in tap water.  $\times 1000$ . FIG. 58. Fifteen minutes after mounting. FIG. 59. Twenty minutes after mounting. FIG. 60. Thirty minutes after mounting. FIG. 61. Thirty-five minutes after mounting.

FIGS. 62-67. Portion of an antheridial filament of *Chara sp.* mounted in once-distilled water.  $\times 1000$ . FIG. 62. Five minutes after mounting. FIG. 63. Ten minutes after mounting. FIG. 64. Fifteen minutes after mounting. FIG. 65. Twenty-five minutes after mounting. FIG. 66. Thirty minutes after mounting. FIG. 67. Forty minutes after mounting.

FIGS. 68-71. Portion of an antheridial filament of *Chara sp.* mounted in one and one-half per cent cane sugar solution.  $\times 1000$ . FIG. 68. Five minutes after

mounting. FIG. 69. Thirty minutes after mounting. FIG. 70. Sixty minutes after mounting. FIG. 71. Two hours after mounting.

FIGS. 72-76. Portion of an antheridial filament of *Chara sp.* mounted in three per cent cane sugar solution.  $\times 1000$ . FIG. 72. Five minutes after mounting. FIG. 73. Fifteen minutes after mounting. FIG. 74. Thirty minutes after mounting. FIG. 75. One hour after mounting. FIG. 76. Two hours after mounting.

FIGS. 77-80. Portion of an antheridial filament of *Chara sp.* mounted in five per cent cane sugar solution.  $\times 1000$ . FIG. 77. Five minutes after mounting. FIG. 78. Twenty minutes after mounting. FIG. 79. One hour after mounting. FIG. 80. Six hours after mounting.

FIG. 81. Portion of an antheridial filament of *Chara sp.* mounted in three per cent cane sugar solution and photographed five minutes later.  $\times 1000$ .

FIG. 82. Same filament transferred to aceto-carmin and photographed fifteen minutes later.  $\times 1000$ .

FIG. 83. Portion of an antheridial filament of *Chara sp.* mounted in three per cent cane sugar solution and photographed ten minutes later.  $\times 1000$ .

FIG. 84. Same filament transferred to aceto-carmin and photographed ten minutes later.  $\times 1000$ .

FIGS. 85-90. Portion of an antheridial filament of *Chara sp.* mounted in aceto-carmin.  $\times 1000$ . FIG. 85. Five minutes after mounting. FIG. 86. Thirty minutes after mounting. FIG. 87. Three hours after mounting. FIG. 88. Six hours after mounting. FIG. 89. Twelve hours after mounting. FIG. 90. Thirty hours after mounting.

### Plate 13

FIG. 91. Terminal end of an antheridial filament of *Chara sp.* mounted in aceto-carmin and photographed one hour after mounting. The appearance of the nuclei suggests the resting stage. The nuclei are more or less uniformly dark, but some differentiated light and dark elements are visible.  $\times 1800$ .

FIG. 92. Four terminal cells of an antheridial filament of *Chara sp.* photographed one-half hour after mounting in aceto-carmin. The nuclei are less uniformly dark than in the previous figure, and show well differentiated dark elements that appear like strands, irregular bodies, and granules. Possibly an early prophase.  $\times 1800$ .

FIG. 93. Four terminal cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in aceto-carmin. The appearance of the dark elements suggests a coarse reticulum lying in an optically hyaline ground substance. Perhaps a later stage than FIG. 92.  $\times 1950$ .

FIG. 94. Terminal cell of an antheridial filament of *Chara sp.* photographed thirty minutes after mounting in aceto-carmin in whose nucleus appears the suggestion of an irregular reticulum lying in an optically homogeneous continuum.  $\times 1000$ .

FIG. 95. Two terminal cells of an antheridial filament of *Chara sp.* photographed forty-five minutes after mounting in aceto-carmin. The appearance of the nuclei suggests possibly a later prophase stage than the previous figures.  $\times 1950$ .

FIG. 96. Two terminal cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. In the terminal nucleus may be counted approximately eleven large, dense elements that appear more or less connected. Obviously a later prophase stage than shown in any of the preceding figures.  $\times 1950$ .

FIG. 97. Terminal cell of an antheridial filament of *Nitella sp.* photographed one and a half hour after mounting in aceto-carmine. There are approximately nine dark, dense and conspicuous elements in the nucleus which appear somewhat twisted and curved, suggesting perhaps chromosomes. Nuclear boundary evidently still present.  $\times 1950$ .

FIG. 98. Terminal cell of an antheridium filament of *Nitella sp.* photographed one hour after mounting in aceto-carmine. Approximately seven more or less connected dense elements are visible.  $\times 1800$ .

FIG. 99. Terminal cell of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. Perhaps a later prophase stage than shown in the previous figure.  $\times 1250$ .

FIG. 100. Three cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in aceto-carmine showing rounded masses of band-like dense elements.  $\times 1250$ .

FIG. 101. Three terminal cells of an antheridial filament of *Nitella sp.* showing three thick, dense bands extending diagonally across the central cell.  $\times 1950$ .

FIG. 102. Terminal cell of an antheridial filament of *Nitella sp.* photographed two hours after mounting in aceto-carmine. In the nucleus may be seen a number of dense chromosome-like elements that appear more or less connected.  $\times 1950$ .

FIG. 103. Terminal cell of an antheridial filament of *Nitella sp.* photographed one hour after mounting in aceto-carmine whose nucleus appears very similar to that shown in the previous figure.  $\times 1950$ .

FIG. 104. Terminal cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in aceto-carmine showing the chromosomes as round bodies. Possibly polar views.  $\times 1950$ .

FIG. 105. Portions of two antheridial filaments of *Nitella sp.* photographed one hour after mounting in aceto-carmine. Equatorial plate shown in cell *A*. The chromosomes marked 2 and 3 in cell *A* show a light central line throughout their length. A similar appearance is visible in the chromosomes marked 1 in cell *B*.  $\times 2400$ .

FIG. 106. Two terminal cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. Nuclei in early anaphase stage. Chromosome groups almost parallel to the long axis of the filament.  $\times 2200$ .

FIG. 107. Late anaphase stage in cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine.  $\times 1800$ .

FIG. 108. Two terminal cells of an antheridial filament of *Chara sp.* photographed twenty minutes after mounting in tap water. Fiber-like and cell plate elements are visible between the daughter nuclei.  $\times 1000$ .

FIG. 109. Late anaphase stage in the terminal cell of an antheridial filament of *Nitella sp.* photographed one hour after mounting in aceto-carmine.  $\times 2200$ .

FIG. 110. Possibly so-called diaster stages in cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in aceto-carmine.  $\times 1800$ .

FIG. 111. Late anaphase stages in cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine in which the daughter groups of chromosomes lie in diagonally opposite corners of the cells.  $\times 1800$ .

FIG. 112. Early telophase stages in two terminal cells of an antheridial filament of *Chara sp.* photographed two hours after mounting in aceto-carmine. The appearance of the nuclei suggests the so-called dispireme stage of fixed and stained preparations.  $\times 1800$ .

FIG. 113. Later telophase stages in cells of an antheridial filament of *Chara sp.* photographed one hour after mounting.  $\times 1800$ .

FIG. 114. Late telophase stages in cells of an antheridial filament of *Chara sp.* photographed one hour after mounting in which cell plate elements are visible between the daughter nuclei.  $\times 1800$ .

FIG. 115. Late telophase stages in cells of an antheridial filament of *Chara sp.* photographed one hour after mounting, in which the nuclei have somewhat the appearance of so-called resting nuclei. The cell walls are completely formed between the daughter nuclei.  $\times 1650$ .





# Persistence of the antipodal tissue in the development of the seed of maize<sup>1</sup>

PAUL WEATHERWAX

(WITH SIX TEXT FIGURES)

It has long been known that the three antipodal cells present at the time of the organization of the embryo sac of maize (*Zea Mays* L.) undergo a series of divisions before the time of fecundation, resulting in the formation of a compact antipodal tissue made up of many cells. The limited number of investigations that have been made indicate that this is a characteristic common to many, if not all, grasses.

Although the function of this antipodal tissue is probably of negligible importance, it is of morphologic interest because it constitutes a gametophytic development unusual in the Angiosperms. The fate of the antipodal cells has usually been disregarded, or disposed of in a few words, in embryological studies of the cereals in recent years, the assumption or the observed fact being that they are early absorbed by the rapidly developing endosperm.

Golinski (1), reviewing the literature of the time of his publication (1893), and adding his own observations, reported that the antipodals were absorbed. He did not have a first-hand knowledge of the embryology of maize, but criticized the work of Westermaier, who reported certain differences between the antipodals of maize and those of such grasses as wheat and barley. Jensen (3) found that the antipodals in wheat were absorbed. Miller (5) reported the same for maize, but two of his figures (*A* and *B*, *pl.* 32) might be otherwise interpreted. Accepting the published views of others, and judging from superficial appearances, the writer (8) has also stated that the antipodals are absorbed in maize.

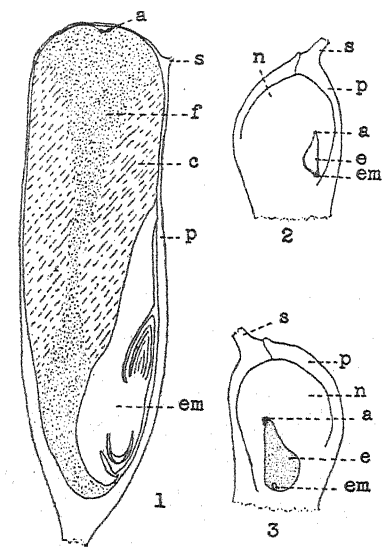
A recent examination, however, of some sections of nearly mature grains of ordinary field corn showed, in the region of the "dent," some small groups of cells distinctly different from those of the nucellus or of the ordinary endosperm (FIGS. 1, 4). This led to a more thorough examination of an old series of

<sup>1</sup> Publication no. 24 of the Waterman Institute for Research.

embryological slides, and of new ones prepared for this purpose, to determine the nature of the tissue in question. It was found that, without doubt, the antipodal tissue often persists to the maturity of the grain. FIGURES 1-6 show the position and appearance of this tissue at different times in the development of the grain. The mature embryo sac has been figured several

times (Weatherwax 7, *fig. 1, pl. 23*; 8, *fig. 13, pl. 7*; Miller 5, *pl. 23*).

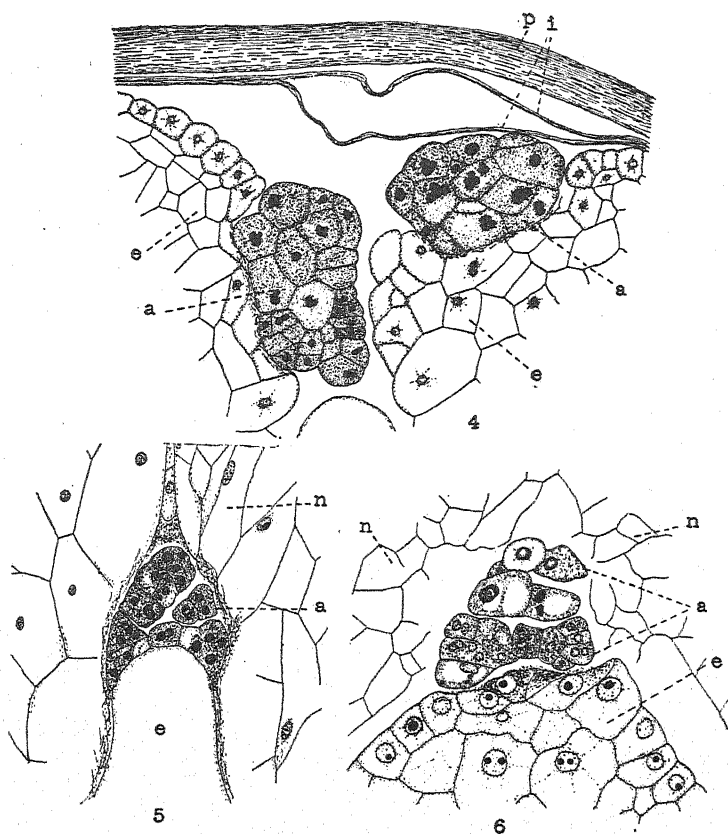
At the time of the maturity of the embryo sac the antipodal cells usually number 25 or more, these having arisen by the division of the original three. In at least the earlier of these divisions the nuclei divide mitotically, but in later stages the division is amitotic, and cells with two, three, or more nuclei are common. After fecundation the antipodal tissue is sometimes soon absorbed by the developing endosperm, but in too many instances for it to be regarded as constituting merely an anomaly, its cells continue to divide and grow slowly at the expense of the impoverished tissue of the nucellus. Occasionally also it may prey upon the



FIGS. 1-3. Longitudinal sections of caryopses of different ages. FIG. 1. Almost mature. FIG. 2. Free-nucleate stage of endosperm development; embryo of only three or four cells. FIG. 3. Intermediate stage of development. *a*, antipodal tissue; *p*, pericarp of development. *a*, antipodal tissue; *p*, pericarp (ovary wall); *n*, nucellus; *e*, endosperm; *f*, floury endosperm; *c*, corneous endosperm; *em*, embryo; *s*, base of style.

nearby cells of the endosperm (FIG. 6). Some of its cells are filled with a dense, deeply-staining protoplasm; others have large vacuoles. The cells are loosely joined together, the one or more groups resembling the loosely organized colonies of some thallophytes. As many as five or six nuclei have been observed in some cells, and fragmentation of the nuclei continues until the seed is practically mature.

Morphologists have pictured the grain of corn as consisting of three distinct generations of tissue: the maternal sporophyte, the embryonic filial sporophyte, and the problematical endo-



FIGS. 4-6. The antipodal masses indicated in FIGS. 1-3, and numbered in the same order. *p*, pericarp; *i*, integument; *a*, antipodal tissue; *e*, endosperm (xeniosphyte); *n*, nucellus; The chalazal end of the young endosperm (*e* in FIG. 5) has a very large vacuole. The apparent disorganization in a few cells at the upper end of the endosperm in FIG. 6 probably indicates at least a temporary invasion of the endosperm by the antipodal tissue.

sperm. To these must now be added the occurrence sometimes of a small portion of the true gametophytic endosperm, the exact homologue of the normal endosperm in most Gymnosperms.

Following fecundation the inside of the grain is the scene of a

life-and-death struggle among these four generations of tissue. The aged and attenuate maternal sporophyte tissue of the nucellus always succumbs, and the young filial sporophyte always wins in the long run, pausing for a while after the maturity of the seed, only to complete its devastation during germination. Possible exceptions may be noted in some of the aberrant types isolated by inbreeding. The two kinds of endosperm, gametophyte and xeniophyte (Trelease 6), compete with varied results, the latter ordinarily persisting to the maturity of the seed, the former sometimes consumed by the xeniophyte, and sometimes persisting, even slightly at the expense of the latter at times. Death of the endosperm doubtless precedes, or is coincident with, the maturity of the seed.

No correlation has thus far been observed between the behavior of the antipodal tissue and the chemical or physical nature of the endosperm of the varieties concerned, but a more detailed study of the endosperm types, including the various "shrunk" and "defective" anomalies (Hutchison 2; Jones 4) might give interesting results.

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# The production of intumescences upon apple twigs by ethylene gas<sup>1</sup>

RAYMOND H. WALLACE

(WITH PLATES 14 AND 15)

## INTRODUCTION

During the past two years I have made numerous experiments on the effect of ethylene gas upon apple twigs and have obtained results which it seems advisable to publish in preliminary form, leaving until later the complete report on the whole subject.

That ethylene induces characteristic reactions in living organisms is well known. Knight and Crocker (1913) showed very clearly that most of the toxic effects of illuminating gas upon plants are probably due to small amounts of ethylene. All of the injurious effects of illuminating gas on vegetation which have been reported in the literature since the time of Girardin (1864) may be due in the greater part to the physiological effects of ethylene. Some of the effects induced by ethylene have been given commercial application during the past few years. Denny (1924) found the yellowing of lemons was greatly hastened by small amounts of ethylene. R. B. Harvey (1925) found it excellent for blanching celery, while Rosa (1925) reports having used it successfully for removing chlorophyll from tomato fruits and for inducing more rapid sprouting in dormant potatoes. Another important application of ethylene is its use as an anaesthetic in surgical practice. From the data reported it is obvious that ethylene is a substance which can induce a wide variety of physiological effects.

The earliest studies on the effect of illuminating gas upon vegetation dealt almost entirely with the killing of trees, shrubs, and herbs, by either accidental or experimental gas leaks. Kny (1871) killed trees by exposing their roots for several weeks to a continuous flow of illuminating gas. Defoliation on the part of the lindens was the first evidence of injury. Späth and Meyer (1873) performed more extensive experiments with a greater number of species and likewise found that defoliation and death results from exposing the roots of trees to gas.

<sup>1</sup> Contributions from the Department of Botany of Columbia University no. 345.

Wehmer (1900) gives perhaps the most striking case on record of gas poisoning of trees. Thirteen elms located on either side of a leaking gas main showed a correlation between intensity of injury and distance from the leak. The effect became evident in late winter when large pieces of bark, in some cases several feet in length, dropped off. Twigs and buds remained normal even on trees whose trunks were completely girdled. During the spring some of the trees developed a complete set of foliage leaves while others did not. He attributed the wide zone of damage to the hard crust on the ground which caused the gas to spread farther beneath the surface than it otherwise would.

The relative susceptibility of various plants to gas poisoning has received no little attention from investigators. Lackner (1873) divided plants into those that are injured and those that are not when grown in a room in which illuminating gas is burned. Neljubow (1901) was able to show that impurities in the air were responsible for the horizontal growth or diageotropism of etiolated pea seedlings so often exhibited by plants grown in the laboratory. He found that either ethylene or acetylene would cause it. He established that diageotropism, increased growth in thickness, and decreased growth in length constituted a triple response to impurities in laboratory air. One part of ethylene to 1,000,000 parts of air gave the response, while one part to 4,000 of air caused the death of the seedlings.

Crocker and Knight (1908) found in the case of carnations that one part of ethylene to 40,000 parts of air killed young buds and prevented the opening of buds already showing petals, while a concentration as low as one part to 2,000,000 caused closing of flowers already open. Using etiolated pea seedlings, Knight and Crocker (1913) found that one part of ethylene to 10,000,000 parts of air prevented elongation of the epicotyl, while four parts to 10,000,000 of air gave the triple response of Neljubow. E. M. Harvey (1915), working in the same laboratory, found that castor bean leaves give a definite nastic response in a concentration of one part of ethylene to 10,000,000 parts of air. The work of Crocker and Knight also indicated that the amount of ethylene present in illuminating gas probably determines the toxic limit of that gas. The responses of carnations, etiolated pea seedlings, and castor bean leaves to ethylene were far more delicate than any possible chemical test for this substance.

Richards and MacDougal (1904) observed no such delicate response in the case of seedlings of *Vicia Faba*, *Zea Mays*, and *Helianthus annuus*, growing in atmospheres composed of about eighty per cent illuminating gas and twenty per cent oxygen. The chief effects noted by them for these high concentrations of illuminating gas were decreased rate of growth, poor differentiation of tissues, retardation of chlorophyll formation, and increase in size of the cells of the cortical parenchyma. These same effects were noted when carbon monoxide was substituted for illuminating gas.

Wehmer (1917) reported water cress as fresh and green after being grown for twenty days in an atmosphere containing twenty per cent of illuminating gas.

Leaf fall, stimulation of root development, and formation of abnormal tissues seem to be common phenomena exhibited by plants as responses to illuminating gas. Thus Kny (1871), Späth and Meyer (1873), Wehmer (1900), Wilcox (1911), and others, as mentioned above, have reported leaf fall for various trees, shrubs, and herbs as a result of accidental or experimental gas poisoning. In some cases leaves turned yellow before falling, while in others there seemed to be only a stimulation of the abscission layer, resulting in the cutting off of the leaf. One of the best examples of defoliation from gas exposure is that given by Wilcox (1911), in which he described the effects of a leaky gas main upon the plants in a commercial greenhouse. The defoliation of the roses was complete, while the effect upon *Coleus* was only slightly less pronounced.

Stimulation of root development was reported by Boehm (1873) for twigs of willow kept in gas-saturated water. Richards and MacDougal (1904) noted a similar effect upon seedlings. Stone (1913) even attempted to use illuminating gas to facilitate the rooting of woody cuttings. He also found a forcing of foliage development, gassed twigs putting out leaves several days before the controls. This is in agreement with the general knowledge of the stimulating effects of low concentrations of poisons upon plants. Some, such as ether and chloroform, have actually been found of value to florists as forcing agents.

A very interesting aspect of the subject is the development of abnormal tissue in plants exposed to ethylene or illuminating gas. There is, however, little agreement as to the tissues affected

and the nature of the proliferations. This lack of agreement may be due to differences in the experimental materials or the experimental methods used by the various workers. Differences in the seasons of the year in which the experiments were made may be of significance.

The development of abnormal tissue is most striking in woody plants, but it is seen also in herbaceous plants and seedlings. Neljubow (1901), in describing the triple response of etiolated pea seedlings, gave increased growth in thickness as one of the effects of ethylene. This response was also observed by Crocker and Knight (1908). Richards and MacDougal (1904) reported a doubling in the size of the cells of the cortical parenchyma in seedlings grown in illuminating gas, as well as a failure in the normal differentiation of the tissues. Substances other than illuminating gas and ethylene have been found to cause proliferations in plants. Grottian (1909) reported that anaesthetics, especially chloroform, caused swellings in roots. Němec (1904) noted similar effects of ether, chloral, benzine, and alcohol vapors.

Woody materials, of both roots and stems, have been found to give rise to proliferations or intumescences under certain conditions. Stone (1913) has described an interesting case of abnormal tissue formation in *Populus deltoides*. The tree, some eight inches in diameter, had been poisoned by gas in the soil and showed on the side of the trunk on which absorption had occurred lesions from  $\frac{1}{2}$  to  $1\frac{1}{2}$  feet long, extending upwards from the level of the soil. At the base of these lesions were pads of delicate parenchymatous tissue from  $\frac{1}{2}$  to  $1\frac{1}{2}$  inches thick that had apparently arisen from the true cambium. The phloem and xylem remained normal in histological appearance. He also described proliferation and swelling for lenticels of willow shoots that had been kept in gas-saturated water.

Gatin (1912), in studying the effect of tarred roads on the adjacent vegetation, found a stimulation of cork formation at the phellogen and a disappearance of the endodermis. The affected trees developed a very small amount of woody tissue, but the phloem remained normal. The cells of the cortical parenchyma were normal in size and form. Analyses of the tarry materials showed the presence of many unsaturated compounds, and he thought that perhaps they were the toxic substances.



Harvey and Rose (1915) tested the effects of illuminating gas and ethylene on root systems. Seedlings of *Catalpa speciosa*, and *Ailanthus*, after exposures of from five to twenty-one days to various concentrations of ethylene or illuminating gas, developed cortical swellings, but similar tests with *Gleditsia* gave no swellings. Both hypertrophy of cortical cells and rapid proliferation of the phellogen layer were said to be involved. When strong streams of gas were passed through soil about the roots of larger potted plants, death ensued in a few days. But when only a small current (about 40 cc. daily) was used, stimulation occurred, resulting in the formation of abnormal cortical tissue on the roots. These abnormalities were reported for *Hibiscus*, *Syringa*, *Diervilla*, *Ricinus*, *Ulmus*, croton and pear.

Doubt (1917) extended the experiments further, using two- or three-year-old trees and allowing the gas of various concentrations to flow around the roots continuously for from fifteen to ninety days. Her results are similar to those of Harvey and Rose. All proliferated tissue was found to be external to the vascular cylinder of the roots, but whether it arose from the cortex or the pericycle was not determined.

Wehmer (1918) obtained results which indicate that trees vary in their sensitiveness to gas poisoning with the time of year. He passed continuous streams of illuminating gas through soil in which were rooted three- to seven-year-old deciduous and evergreen trees. In the spring they were killed in a short time, as was the case with herbaceous plants at all times. In late summer and early fall the trees were defoliated. In the dormant winter condition the trees gave no apparent response. He mentions no abnormal tissue formations such as those found by Stone, Gatin, Harvey and Rose, and others.

Woffenden and Priestley (1924) exposed elder plants to constant streams of illuminating gas by encasing the stems in tubes and passing gas through the tubes. The reaction obtained was found to vary with the stage of development of the stems. Young apical regions were killed in a few hours, older internodes directly below showed intumescences in the lenticels. Portions lower than the fifth node became discolored and swollen. The experiments, begun on July 1, were discontinued on August 25, because after that time no reaction could be obtained. The cessation of the capacity to react is attributed to the completion

of the cork layer which occurred about this time. They attribute the increased rate of cell division to increased permeability of the cells of the phloem and cortex, and suggest that the unsaturated linkages of the gas compounds unite with those of the unsaturated fatty acids in the cells, and thus give rise to more diffusible compounds, which spread outward and leave the whole cortex more permeable to water and nutrient materials. They sum up the work on woody stems in the following manner:

Earlier work upon the effect of coal gas upon plants does not always provide sufficient anatomical data for exact comparisons, but there can be little doubt that where these observers record effects upon the bark of woody plants their results admit of explanation in the light of the experiments described above, and in every case are due to cells arising from the cork phellogen originally proliferating and failing to suberize.

Harvey and Rose (1915) mention hypertrophy of cortical cells and activity of the phellogen as being responsible for the abnormal tissue. They give figures for *Catalpa*, *Ailanthus*, *Syringa* and *Hibiscus*, the first three of which indicate cortical division unless the phellogen cuts off cells internally rather than externally. For *Hibiscus*, on the other hand, the figure shows the proliferation to be definitely phellogenic in nature. They fail to distinguish between the two types of figures. Doubt (1917) says all proliferation is external to the vascular cylinder, but does not know whether it arises in the pericycle or cortex. She makes no mention of the phellogen. In the case of the proliferated pads of parenchymatous tissue reported by Stone (1913) for *Populus deltoides*, it is physically impossible for it to have arisen from the cork cambium since phellogen could not give rise to tissue lying between xylem and phloem. From the results reported in the literature it is therefore evident that although the phellogen in many cases appears to be the reacting tissue, nevertheless in other cases proliferation may occur in other tissues.

#### MATERIALS AND METHOD OF EXPERIMENTATION

My studies have so far been confined entirely to woody stems and have extended over two consecutive years, chiefly in the fall and winter period from September to March. The material for the experiments consisted of branches from 1 to 1.5 cm. in diameter cut from trees or shrubs growing out of doors. The

shoots were then cut into short lengths (10 to 15 cm. long) and the latter were grouped into sets so that each set contained a graded series of sizes from 1.5 cm. in diameter down to slender terminal twigs. These sets were placed in belljars with the basal end of each piece in moist sand as if for rooting experiments. A measured volume of ethylene was liberated in each jar and left for twenty-four to forty-eight hours, after which the jars were opened for twenty-four hours to allow all the ethylene gas to escape. The jars were then left closed except when observations were being made. A similar set of control twigs, receiving identical treatment except for the absence of the ethylene, was provided with each experiment.

The reactions observed in this study are those which may be obtained with woody twigs collected in the season of the year during which physiological processes are the least active. Rather low concentrations of ethylene for a single rather short period were used. After the exposure to the gas, the twigs remained in a water-saturated atmosphere, free from the gas. It was found that the reaction might take place within a few days after the exposure to the gas, or it might require several weeks.

#### GENERAL REACTIONS

Twigs in the controls showed only slight responses to the higher temperatures and humidity of the environment provided. In one experiment which may be taken as typical of many others, enlargement of the lenticels occurred in only three species out of fifty within three weeks after the experiment was begun. Aerial roots appeared in four species (*Populus candicans*, *Salix purpurea*, *S. pentandra*, and *S. vitellina*). Only a few species were found to leaf out in experiments made during fall and early winter, but during late winter and early spring many species developed complete series of leaves. Callus formation at the basal end of the twigs occurred in more than fifty per cent of the species tested. Among the twenty-eight that formed callus, eleven showed approximately equal development of callus on both the morphologically apical and morphologically basal ends, while the remaining seventeen formed it only on the basal ends.

Many of the responses reported for plants in general have been found to occur in woody stems following the gas treatment outlined above. Apple twigs which were tested early in the

autumn before the leaves had fallen showed defoliation within two or three days, the leaves remaining green. Stimulation of root formation resulted in many plants but was more pronounced at certain seasons than at others. Proliferation of cells in the lenticels occurred in *Sambucus*, *Syringa*, *Catalpa*, and others.

Forcing of buds was observed in almost every species tested in this study. Buds may be forced at almost any season. A rather interesting relation has been noted in this connection. In the early winter the resulting leaves are more or less abnormal, appearing as if burned on the edges, while later (usually early in February) the buds may produce healthy leaves.

Although ethylene has been used to cause the disappearance of chlorophyll from certain plant parts (such as celery leaves and tomato fruits), it appears to produce no such effect on the chlorophyll of the chlorenchyma of woody stems. I have frequently observed cases in which the cork was disrupted and the green layers below were exposed, but in no case have I observed an apparent decrease in the chlorophyll content of the cells. This is true both for experiments made in darkness and in light.

#### THE FORMATION OF INTUMESCENCES IN APPLE TWIGS

It is well known that hypertrophy may occur in the tissues of stems and roots when they have been exposed to stimulating agents. *Gymnosporangium* galls, crown galls, etc., arise from parasitic stimulation of the host tissues. In the former case, Stewart (1915) stated that it is proliferation of an axillary bud that gives rise to the gall. Smith (1917) was able to simulate closely crown gall formation on *Ricinus communis* by injecting various substances into the plant, and concluded from this evidence that crown galls are formed in response to chemical stimuli by the substances which are by-products of the life activities of *Bacterium tumefaciens*. Hahn, Hartley, and Rhoads (1921) report hypertrophied lenticels on the roots of conifers that had been grown in excessively moist soil. Plants growing in bog water are reported commonly as having abnormal roots. The work of Harvey and Rose (1915) and Doubt (1917) has shown clearly that many woody plants will develop proliferated cells in the cortical region if illuminating gas or ethylene is passed through the soil around the roots.

Apple stems, when in the most sensitive condition, may show,

as a result of ethylene stimulation, intumescences in their buds, at either their basal or apical ends, or in their internodal regions. Of the three responses, that in the buds is the most striking and so far as I can find has never been reported for any plant. The reaction appears first as a transverse breaking of the epidermis and cork layers on each side of the bud base (FIG. 3). The actual time required for the appearance of the reaction is in some cases as low as forty-eight hours, while in others as long as six days may intervene between stimulus and reaction. In general about four days is required. Proliferation of the cells of the bud and bud base may proceed very rapidly and within three or four days the entire resting buds, containing the leaf primordia for the following spring, may be completely destroyed. The tissues of the buds, some of which already are highly differentiated, apparently revert to an embryonic condition and the resulting mass of living cells has a loose granular appearance. This reaction in the buds extends to the xylem of the stem. Externally the buds at this time present an appearance very suggestive of popped corn (FIG. 1). The cork layer, bud scales, etc., are curled back, while the soft, almost white, proliferated cells protrude. A slight jar often suffices to cause a mass of the proliferated tissue to fall away from the stem. Not infrequently the same kind of reaction occurs in the region of bud-scale scars which mark the position of the terminal bud produced at the end of some previous year's growth (FIG. 4).

Terminal swellings usually begin before the bud reactions are complete; that is, from five to eight days after exposure to the gas. Terminal swellings are very common on cut ends of twigs, but in some cases appear also on the uncut ends of short lateral branches. They first become evident by a breaking of the epidermis near the tip of the twig (FIG. 5), due to an enlargement of the tissue beneath. For several days they may remain as slight swellings in the bark, but within a week they may become very pronounced, in many cases increasing by 6 or 8 mm. the normal diameter of the twig. This occurs even in the case of twigs only 4 mm. in normal diameter (FIGS. 6, 7). As may be seen in the figures, the line between normal and swollen tissue is very sharp.

The internodal swellings occur later than the terminal swellings, appearing in from nine to twelve days after exposure

to the gas and reaching their maximum development five to ten days later. The internodal swellings seem to be little correlated with either the bud or the terminal reactions, since they often occur when neither of the others is present (FIGS. 10, 11). The exact depth in the bark to which the terminal and internodal swellings penetrate has not been definitely determined, but there appears to be in many cases a combined hypertrophy and hyperplasia of both phloem and cortex. This phase of the problem, as well as the cytological data relating to the behavior of the cell structures, is to be discussed in my full report.

Although bud destruction, terminal swelling, and internodal swelling constitute the typical series of reactions when the twigs are most responsive, intumescences of the buds may not occur, while terminal and internodal swellings follow in their regular sequence.

#### EFFECT OF ENVIRONMENTAL CONDITIONS ON THE REACTIONS OF APPLE TWIGS

Experiments on the relationship of external conditions to the response above described have given some interesting suggestions. For the present I shall give only a summary of my experiments, leaving until later the detailed statistical treatment of the data. The statements are, in every case, based upon concordant results from several experiments.

*Concentration and presentation time.* Concentrations greater than one part of ethylene to 4,000 parts of air showed little or no increased intensity of reaction. Identical responses in all three regions were obtained with a series of concentrations ranging from as low as one part of ethylene to 4,000 parts of air to as high as one part of ethylene to seven of air. Exposures of one, two, and ten days gave identical results in buds and terminal intumescences; with the longer periods there was a doubtful increase in the degree of internodal swelling. Further experiments are being made with low concentrations and shorter presentation times.

*Temperature.* All experiments were made in greenhouses with a mean temperature of about 21° C. and a daily range of from 10 to 15° C. I have collected twigs at a widely varying range of temperatures. Material was collected before frost

in the fall as well as at  $-20^{\circ}$  F. in December. Pronounced intumescences were obtained in buds, ends of twigs, and internodes in material collected at all these temperatures.

*Wounding as an aid to penetration by the gas.* Readiness of penetration of the gas into a tissue might affect the time and degree of response of the tissue, all other conditions being constant. Experiments designed to test this factor, however, showed negative results in all cases. Scraping, notching, or slitting of the bark of the twigs before they were exposed to the gas had no apparent influence on the responses. Buds which were covered with moist sand, owing to their position near the lower end of the stems, reacted as did those above in the air. Inverting the twigs brought out an interesting result. The buds and internodes react as do those of twigs in normal position; the morphologically apical end now being downward does not react, while the morphologically basal end which is above does react. The terminal swelling may, therefore, be obtained from either the morphologically apical or basal end according to its position.

*Species of plants which will react.* Forty-seven species of woody plants were tested, and they have shown that it is rather rare for intumescences to be formed in all three regions, buds, internodes, and apices of the same plant. A cultivated crab (*Pyrus Malus*), Transparent apple (*P. Malus*), *P. ioensis*, and *Ginkgo biloba* each gave responses in the three loci. Certain other kinds of stems showed intumescences in only one or two of the regions: *Populus deltoides* (FIG. 2), *P. tremuloides*, and *Salix longifolia* gave responses in the buds and also gave internodal swellings, but no terminal swellings. *Syringa*, *Forsythia*, *Prunus virginiana*, and others gave internodal swellings, but no bud response or terminal swellings. A relatively large number, sixteen in all, including *Sambucus canadensis*, *Acer Negundo*, *Juglans cinerea*, etc., showed a stimulation of the lenticels, but failed to show any response in buds, apices, or internodes. Approximately fifty per cent of the species tested showed no visible response in the form of intumescences when treated with ethylene gas.

#### EFFECT OF ETHYLENE UPON CALLUS FORMATION

In the course of my experiments on the effect of ethylene upon stems it was observed that there was an apparent inhibition

of callus formation, though in general the effect of ethylene on twigs seems to be to stimulate cell division in some region or other. Experiments showed conclusively, however, that ethylene may inhibit the development of callus. Twigs from forty-seven species of trees and shrubs growing out of doors were cut on November 6 and placed in moist chambers. Twenty-eight had developed callus by December 14. Eleven of these species without gas treatment showed what might be called good callus, while twigs of the same kind treated for forty-eight hours with concentrations of 1-400 and 1-1600 showed no callus at all. In fourteen cases some callus was present on the treated twigs, generally in the lower concentration, but the callus on the untreated twigs was better developed. In the three remaining species the callus was apparently equally developed on both treated and untreated twigs; but in no case was it better developed on the treated twigs than on those not treated. A repetition of the experiment, using twigs cut from the same trees on January 26, showed on February 7 results concordant with those of the first experiment.

#### GENERAL DISCUSSION

I have not determined just how and where these intumescences arise in the tissues of the apple. The pads of parenchymatous tissue described by Stone (1913) for *Populus deltoides* seem from both the text and figures to be cambial in nature. Harvey and Rose (1915) and Doubt (1917) are very specific in stating that the swelling was external to the vascular cylinder in the woody plants studied by them. The apple intumescences in the buds, in the apices, and in the internodes seem to involve proliferation of tissues within the vascular cylinder as well as external to it. The bud intumescences are so striking in appearance that had any of the earlier students of gas effects seen them their description could leave no doubt as to the identity of the reactions.

The lowness of the concentration and the shortness of the presentation time which were found sufficient to bring about a response raise some interesting questions. The lowest concentration and the shortest presentation time so far used were one part of ethylene to 5,000 parts of air for twenty-four hours and one part of ethylene to 400 parts of air for two hours; and under



these conditions definite intumescences in the three loci developed. Harvey and Rose (1915) and Doubt (1917) used long exposures to gas in their experiments on woody plants, none being for less than five days and some being for as long as three months. It would be interesting to know if similar responses would have resulted from presentation times of twenty-four hours or less. This would seem probable if the reactions are analogous to those I have found in apple twigs.

As previously mentioned, Denny, Harvey, and others have caused chlorophyll to disappear from certain plant parts by exposing them to ethylene. The chlorophyll content of the chlorenchyma of woody stems, however, was not visibly diminished by ethylene treatment, under the conditions of the present experiments. FIGURES 6 and 7 show two terminal swellings on Transparent apple twigs and in each case the cork layer has peeled back, exposing the chlorenchyma layer. In FIGURE 7 the chlorenchyma has split, exposing the white cortical and phloem tissues beneath. The dark shade of the chlorenchyma indicates that chlorophyll is still present. It should be pointed out in this connection that the disappearance of chlorophyll in lemon fruits and celery stalks is merely hastened by exposure to ethylene gas.

The apparent inhibition of callus formation by ethylene is exceptional, since many of the responses of plants to gas seem to be definite cases of stimulation of cell division. Experiments in which concentrations of one part of ethylene to 400 or 1600 parts of air were used showed inhibition for at least eighty per cent of the species that normally formed callus in the control. In many cases callus inhibition was the only evidence of injury that the twigs exhibited. Often, however, callus inhibition was found associated with another response to ethylene, the development of intumescences in the lenticels. FIGURES 12 and 13 show this association for *Sambucus* (FIGURE 12 shows control twigs, while FIGURE 13 shows the treated). Further experiments are in progress, testing lower concentrations and shorter presentation times, in an attempt to find a concentration or presentation time that may stimulate callus formation.

## SUMMARY

Experiments on woody twigs conducted for two consecutive years, chiefly from September to March, showed many cases of intumescence formation in response to ethylene stimulation. The reactions were obtained by exposing cut twigs of various trees and shrubs to a known concentration of ethylene for a single period (from two to 48 hours), after which the twigs were kept in a saturated atmosphere free from the gas.

Different species varied greatly in their response to ethylene stimulation. The most pronounced type of intumescence, involving the destruction of buds, swelling of the apices and internodes, was obtained in *Pyrus Malus* (varieties Transparent apple and cultivated crab), *P. ioensis*, and *Ginkgo biloba*.

Concentrations greater than one part of ethylene to 4,000 parts of air showed little or no increased intensity of reaction. Identical responses occurred in concentrations ranging from 1-7 and 1-4,000.

Although a presentation time of forty-eight hours was used in most of the experiments, a presentation time of two hours gave a response.

Ethylene had no apparent effect on the chlorophyll in the chlorenchyma of woody stems.

At least eighty per cent of the twigs which normally formed callus in the controls showed an inhibition of callus formation after an exposure to a low concentration of ethylene gas.

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## Description of plates 14 and 15

## PLATE 14

FIG. 1. Buds of *Pyrus Malus*, var. Transparent ( $\times 1.5$ ), five days after exposure to ethylene. Buds have formed intumescences; lenticels are normal.

FIG. 2. Twig of *Populus deltoides* ( $\times 2$ ) six days after exposure. Buds are raised upon a pad of proliferated tissue, which is characteristic of poplar and willow.

FIG. 3. Side view of reacting Transparent apple bud ( $\times 4$ ). Reaction is incomplete.

FIG. 4. Intumescence in a terminal bud scar ( $\times 2$ ) marking the end of a previous year's growth (Transparent apple).

FIG. 5. Transparent apple twig ( $\times 2$ ) ten days after exposure, showing completed intumescence in a bud and the beginning of a terminal swelling.

FIG. 6. Same twig as shown in FIG. 5 but one week later, showing completed terminal swelling. Lesions in the chlorenchyma layer expose the white cortical tissue beneath.

FIG. 7. Transparent apple twig ( $\times 2$ ) ten days after exposure. Note the splitting of the cork and chlorenchyma layers, exposing the deeper tissues below. The contrast of light and dark indicate that chlorophyll is still present.

## PLATE 15

FIG. 8. Transparent apple twig ( $\times 2$ ) with terminal intumescence and slight indications of internodal intumescence ten days after exposure to ethylene. Note the intumescence in the terminal bud on the small lateral branch.

FIG. 9. Same twig as shown in FIG. 8 one week later, showing intumescences in the terminal bud, apex, and internode of the same twig.

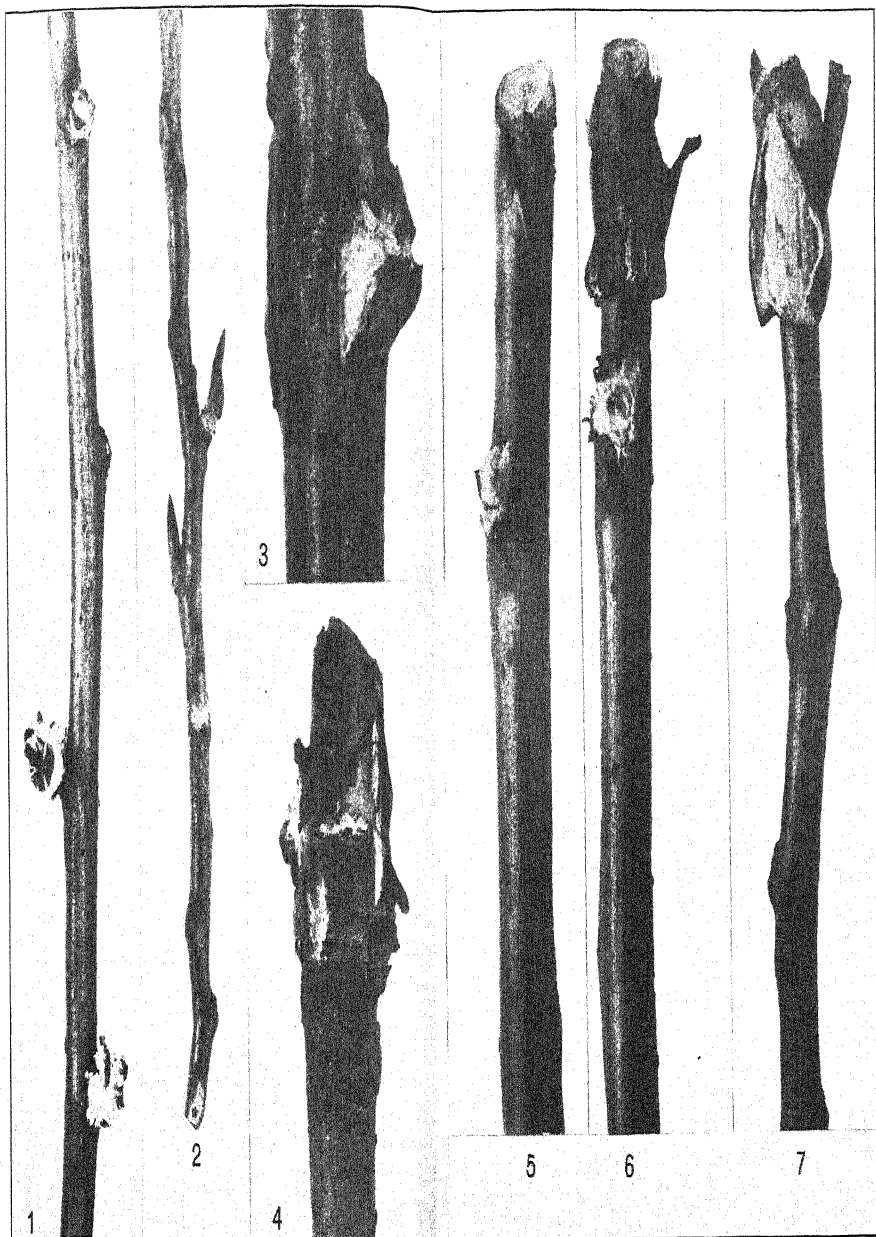
FIG. 10. Twig of *Pyrus ioensis* ( $\times 1$ ) showing normal internode.

FIG. 11. Twig of *Pyrus ioensis* ( $\times 1$ ) showing internodal swelling, but normal bud and apex.

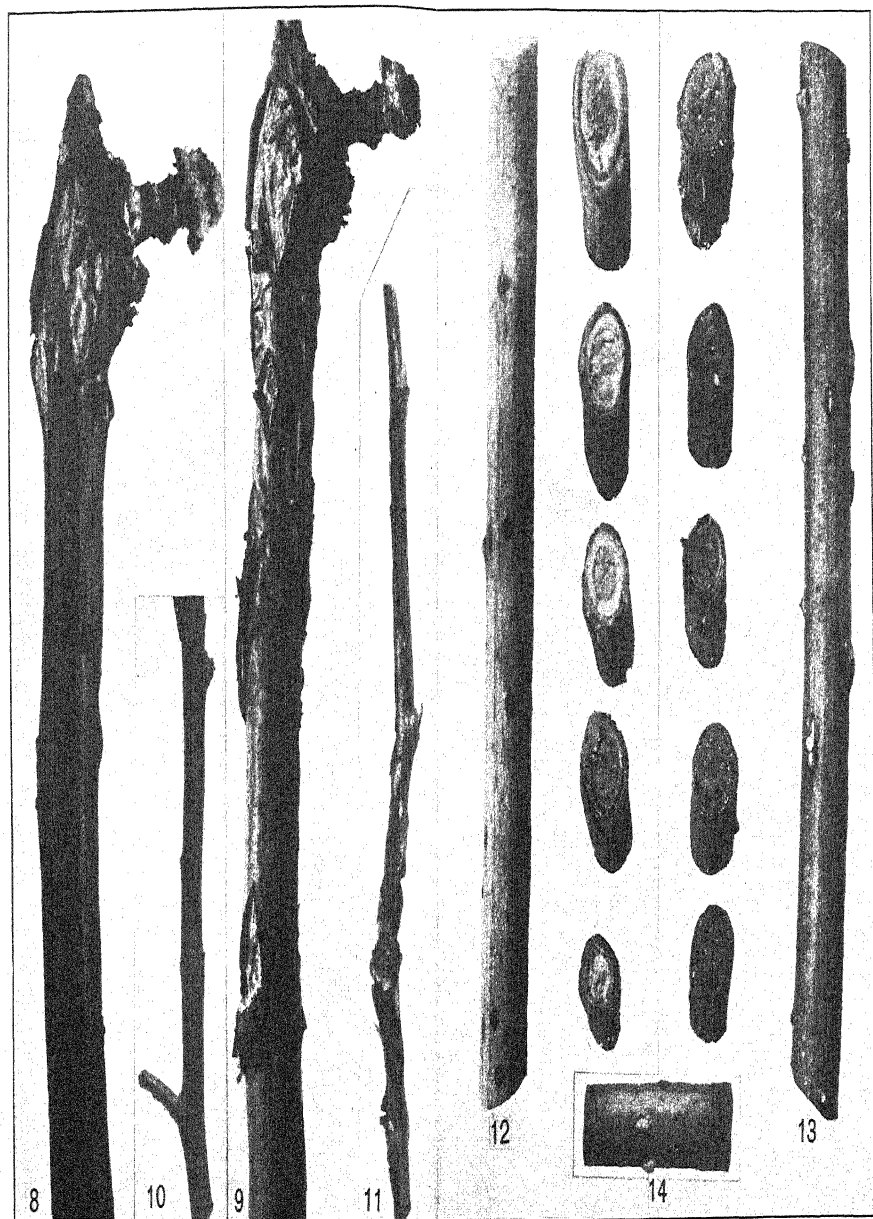
FIG. 12. *Sambucus* twigs ( $\times 1$ ) from control, nineteen days after the experiment was begun. Good callus has developed and lenticels are normal.

FIG. 13. *Sambucus* twigs ( $\times 1$ ) nineteen days after exposure to the ethylene. The lenticels are enlarged and no callus has developed.

FIG. 14. Enlarged lenticels of *Sambucus* from ethylene jar.



WALLACE: INTUMESCENCES ON TWIGS



WALLACE: INTUMESCENCES ON TWIGS





## Notes on the flora of the Miocene of the Tesla region, California

FLORA MURRAY SCOTT

(WITH PLATE 16)

From the upper San Pablo (Miocene) of the Tesla region, Alameda County, during the summer of 1922, a small collection of fossil leaves was obtained; the specimens are deposited in the collection of Stanford University. The impressions are well preserved in fine grained sandstones and calcareous sandstones; they are occasionally partially carbonized. They represent the detritus of a temperate, well watered area with a rich and varied growth of broad-leaved trees.

The genera identified, besides one monocotyledonous specimen, are distributed through the following dicotyledonous families: Salicaceae, Fagaceae, Lauraceae, Platanaceae, Rosaceae, Aceraceae, Cornaceae, and Oleaceae.

### DETAILED DESCRIPTION

The Tesla flora comprises 18 genera, of which 13 species have been indentified. The specimens will now be discussed in detail.

1. A monocotyledonous species. A small, but recognizable, fragment of a monocotyledonous leaf of the type of *Typha*.

2. *POPULUS ALBA* L. (?) Two leaf fragments of the genus *Populus* do not appear to be referable to any fossil species so far described from the Pacific Coast. The broad apex and widely crenate margin of the leaves are in marked contrast to the narrow or acuminate apex, and closely crenate or almost dentate margin of the majority of fossil and present day forms. These characters, however, are diagnostic of the European species *Populus alba*. The only fossil species which the specimens in question at all resemble is *Populus polymorpha* Newb., described by Newberry from Bridge Creek, Oregon. Two fragments, however, hardly constitute sufficient foundation on which to base a theory of the former distribution of *Populus alba*, nor considering the variability of leaves, is it advisable to attempt to delimit a new fossil species.

3. *POPULUS* sp. Another leaf fragment is referable to the genus *Populus*, in finely serrate margin, venation, and broadly ovate shape, showing a close resemblance to such a type as *Populus tremuloides* Michx.

4. *SALIX* sp. The genus *Salix* is represented by one small and perfect leaf and a number of fragments.

5. *CORYLUS* sp. One specimen shows the basal part of the leaf with the unmistakable form and venation of this genus.

6. *ALNUS RHOMBIFOLIA* Nutt. From the numerous, but unfortunately incomplete fragments of *Alnus*, it is seen that, when entire, the leaves are 3-5 cm. broad, and 5-6 cm. in length. The venation is well preserved, the tertiaries being particularly distinct. The leaf margin is slightly doubly serrate.

*Alnus corrallina* Lesq. from Corral Hollow is described as follows (Lesquereux 5):

Leaves oblong-ovate, thickish, rounded in narrowing to a short petiole, obtusely pointed, doubly denticulate; teeth short, acute, turned outside, glandulose; secondary nerves close, parallel, straight to the borders, branching in the upper part; nervilles distinct, close, simple, rarely branching, at right angles to the veins; catkins . . .

From this description and from the figures which accompany it, the present leaf impressions differ only in their slightly larger size, a point of minor importance in leaf identification.

Lesquereux remarks on the close affinity of *Alnus corrallina* with the present day *Alnus viridis* of the eastern states. On comparing the present fossils with herbarium material, it is seen that, from a consideration of leaf alone, it is impossible to distinguish between the Tesla leaf impressions and the following species: *A. viridis*, *A. rhombifolia* and *A. rugosa*. Age and habitat may accentuate certain variations in size, in leaf margin, and in the prominence of the tertiary veins. This last character is perhaps most constant in *A. rhombifolia* Nutt., one of the commonest alders of the Pacific Coast. In short, *Alnus rhombifolia* Nutt. appears to be identical with *Alnus corrallina* Lesq., and may therefore be regarded as dating back to Miocene.

7. *QUERCUS KELLOGGII* Newb. One fragment of a large oak leaf, 5.5 cm. broad and probably 12 cm. long when complete, resembles very closely the California Black Oak, *Q. Kelloggii*. *Quercus pseudo-lyrata* Lesq., a fossil species from the John Day

Basin, in form and in venation bears a striking resemblance both to the Tesla fossil in question, and to the present day leaf of *Q. Kelloggii*, a species which ranges from Oregon to San Diego.

8. *QUERCUS CHRYSOLEPIS* Lebm. Another species of *Quercus* is present in a small but perfect leaf, that is not to be distinguished from *Q. chrysolepis*, the present common Canyon Oak of the Pacific Coast.

9. *CASTANEA* sp. Two small fragments in venation and in leaf margin show a very close resemblance to the modern chestnuts, in particular to the species *Castanea pumila* (L.) Mill.

The family Lauraceae is important in the Tesla flora, being represented by no less than seven forms, belonging to the genera *Laurus*, *Persea*, *Umbellularia*, and *Cinnamomum*.

10. *LAURUS GRANDIS* Lesq.; 11. *LAURUS PRINCEPS* Heer; 12. *LAURUS* sp. The two fossil species recorded by Lesquereux (5) from Corral Hollow, *L. grandis* and *L. princeps*, are identifiable in a few large and well preserved fragments. A single leaf fragment, elliptical in outline, coriaceous, and pinnately veined, may also be referred to the genus *Laurus*.

The entire absence of this genus from North America today is interesting in view of its previous very wide distribution. Of present day forms the species *Asimina triloba* Dunal shows the closest resemblance in venation to the fossil species of the Lauraceae.

13. *PERSEA BORBONIA* Spreng., and 14. *PERSEA ALPIGENA* Spreng. (FIG. 2) *Persea*, a tropical and subtropical genus of the American continent, fairly common in the Southeastern States, at one time appears to have flourished on the Pacific Coast. Numerous leaves in the Tesla formation are not to be distinguished from present species of the former region, viz., *P. Borbonia* Spreng. and *P. alpigena* Spreng.

15. *UMBELLULARIA CALIFORNICA* Nutt. The sole and by no means unimportant member of the family Lauraceae on the Pacific Coast today, *Umbellularia californica* Nutt., is recognized in one fragmentary fossil. While bearing a certain resemblance, especially in the form of the leaf, to *Salix lasiolepis* Benth., the more leathery texture, and in particular the fine and very distinct reticulation, of *Umbellularia* appear to be diagnostic.

16. CINNAMOMUM sp. The tri-nervation of one widely ovate-elliptical fossil leaf refers it to the genus *Cinnamomum*.

17. PLATANUS DISSECTA Lesq. A great part of the leaves of the Tesla collection appear to belong to the genus *Platanus*. The leaves of *Platanus dissecta*, first described by Lesquereux from Corral Hollow, are extraordinarily well preserved, though unfortunately they are in all cases incomplete. From the very numerous fragments, however, a fairly complete reconstruction is possible.

Along with the leaves are found many well preserved pieces of rugose bark (FIG. 4). This appears to be identical with the bark of the present day form, *Platanus racemosa* Nutt. In leaf also, *Platanus dissecta* very closely resembles the modern species. A point of difference is seen in the leaf margin. This, in *Platanus racemosa*, is usually entire. In one specimen from Colusa County, California, however, the margin is toothed, differing in no wise from the margin of the fossil species.

18. PRUNUS DEMISSA Walp. (FIG. 1). Very numerous leaves, both complete and fragmentary, of the genus *Prunus*, are not referable to any Pacific fossil species, from all of which they appear to differ in the nature of the secondary veins. These, in the majority of fossils, are inconspicuous, and disappear towards the leaf margin. In the Tesla specimens, however, they are well marked, somewhat irregular, and arcuate towards the margin; the venation is in brief similar to that seen in the present day *Prunus demissa* Walp. The leaf margin is not always distinguishable in the fossils, but a few specimens show a minute serration like that of the present form.

19. ACER sp. Two specimens, at first included along with the Platanaceae, may possibly represent the genus *Acer*. They are unfortunately incomplete, but show a very close resemblance to the fossil species *Acer Osmondi* Knowlton, and also to the present day sugar maple, *A. saccharum* Marshall.

20. CORNUS NUTTALLII Aud. One leaf fragment resembles very closely the present Pacific dogwood, *Cornus Nuttallii*.

21. FRAXINUS OREGONA Nutt. (FIG. 3). Ash leaflets, identical with the present day species *Fraxinus oregona*, are abundant and perfectly preserved. In one specimen the leaflets are seen to be nearly sessile.

*Fraxinus oregona* differs in certain characteristics from the

Pacific Coast fossils already described from the Clarno formation, viz. *Fraxinus oregonensis* Knowlton and Cockerell, and *F. denticulata* Heer. The Alaskan form *Fraxinus heerendensis*, while closely resembling the Tesla species, is distinguished by the toothed leaf-margin.

22. *CHRYSOPHYLLUM* sp. A fragmentary leaf, conspicuous in the right angled origin of the closely set parallel secondary veins, bears a striking resemblance to the genus *Chrysophyllum* and in particular to the Porto Rican species *Chrysophyllum Cainito* L. *Chrysophyllum* today is a tropical and subtropical genus, being represented by a few species only in the South-eastern States. It does not occur on the Pacific Coast.

#### COMPARISON WITH OTHER FLORAS

The two main Miocene floras of the Pacific Coast with which the Tesla flora may be compared are: (1) flora of the John Day Basin (Mascall) Oregon; (2) flora of the Auriferous Gravels (Knowlton 2, 3). On comparing the Tesla fossil list with the former, it is seen that, with the exception of *Laurus grandis* Lesq., *Laurus princeps* Heer, *Platanus dissecta* Lesq., the Tesla species are absent from the Mascall formation; nor are the following genera represented:—*Umbellularia*, *Cinnamomum*, *Fraxinus*, *Chrysophyllum*. Similarly from the Auriferous Gravels the following fossil species occurring in the Tesla beds are reported:—*Laurus grandis* Lesq., *Laurus princeps* Heer., *Platanus dissecta* Lesq.; while the genera *Alnus*, *Umbellularia*, *Cinnamomum*, and *Chrysophyllum* do not occur.

On the other hand the fossil species of *Aralia*, *Artocarpus*, *Ficus*, *Liquidambar*, and *Magnolia*, common to the Mascall formation, and to the Auriferous Gravels, do not appear in the Tesla formation. In short, in genera and species the Tesla flora is strikingly modern.

The distribution of the modern forms is interesting. The majority are common on the Pacific Coast. Three species, however, do not occur west of the Continental Divide, viz., *Populus alba* L., *Persea Borbonia* Spreng., and *P. alpigena* Spreng. *Populus alba* L. is today a European form. The identification of this species here rests, as already stated, on somewhat scanty material, and therefore, pending further fossil evidence, it may temporarily be disregarded. The genus *Persea* today ranges

along the Coastal belt from the Southeastern States as far south as Brazil and Chile, i. e., it is a tropical and subtropical genus.

The presence of the genera *Persea*, *Chrysophyllum*, and *Cinnamomum*, which have survived from the definitely subtropical Eocene, may be taken to indicate that, during the time of the Tesla deposition, conditions slightly warmer than those of the present day obtained; while the absence of *Aralia*, *Artocarpus*, *Ficus*, *Liquidambar*, and *Magnolia*, of the Mascall formation and of the Auriferous Gravels, genera today typical of subtropical regions, is significant of the changing climate.

#### GENERAL SUMMARY

The preponderance of present day species in an Upper Miocene flora appears, in comparison with other floras of the same age, to be somewhat unusual. When, in the process of identification, herbarium material is examined, the extraordinary variability of leaf forms is at once apparent. A complete and continuous series of variations can be traced in such characters as the following: the size and shape of the leaf, the angle of the apex, the nature of the leaf margin. One thing only remains relatively constant, and that is the venation. The ultimate branching of the veins, determining the leaf areolation, may also be included in this category, but this finer reticulation is not always preserved in the fossil state. Even in these last characters, however, modifications constantly occur. Age and habitat may not only obliterate distinctions but may impose thereon resemblances entirely illusory. From a leaf alone accurate specific determination appears to be impossible. This fundamental capacity for leaf-variation must be considered when dealing with a fossil flora. For this reason, it appeared to be impossible to delimit the Tesla fossils from the present day species. In all cases the leaf impressions showed a closer resemblance to modern forms, as represented in herbarium material, than to any fossil species hitherto described.

Should this same fact emerge from a consideration of other fossil floras, then the present ideas on the longevity of plant species will require to be somewhat revised.

#### CONCLUSIONS

1. The Tesla flora is broad-leaved in character, the flora of a moist and temperate region.

2. That the climate may have been slightly warmer than today at the time of Tesla deposition is suggested by the presence of the at least subtropical genera *Persea*, *Cinnamomum*, and *Chrysophyllum*.

3. The Tesla flora differs from other fossil floras of a like age in its exceedingly modern aspect.

4. Certain present day species may be regarded as dating back to upper Miocene.

5. The absence of the genus *Laurus* from the present day flora of North America is so far unexplained. The conditions which brought about its restriction and ultimate extinction are unknown. A general resemblance to the genus *Asimina* is noted.

6. The fossil species *Alnus corrallina* Lesq. does not appear to differ from the present *Alnus rhombifolia* Nutt.

7. Considering the wide range of fossil species the use of a fossil flora in detailed stratigraphy is therefore seen to be practically nil.

The main interest of the fossil plant record lies in evidence it affords regarding former climatic conditions, and in these so called "fossil" climates lies a key to the present day distribution of plants.

I have pleasure in expressing my thanks to Dr. J. P. Smith and Dr. LeRoy Abrams for their criticism and assistance in the identification of the above fossils.

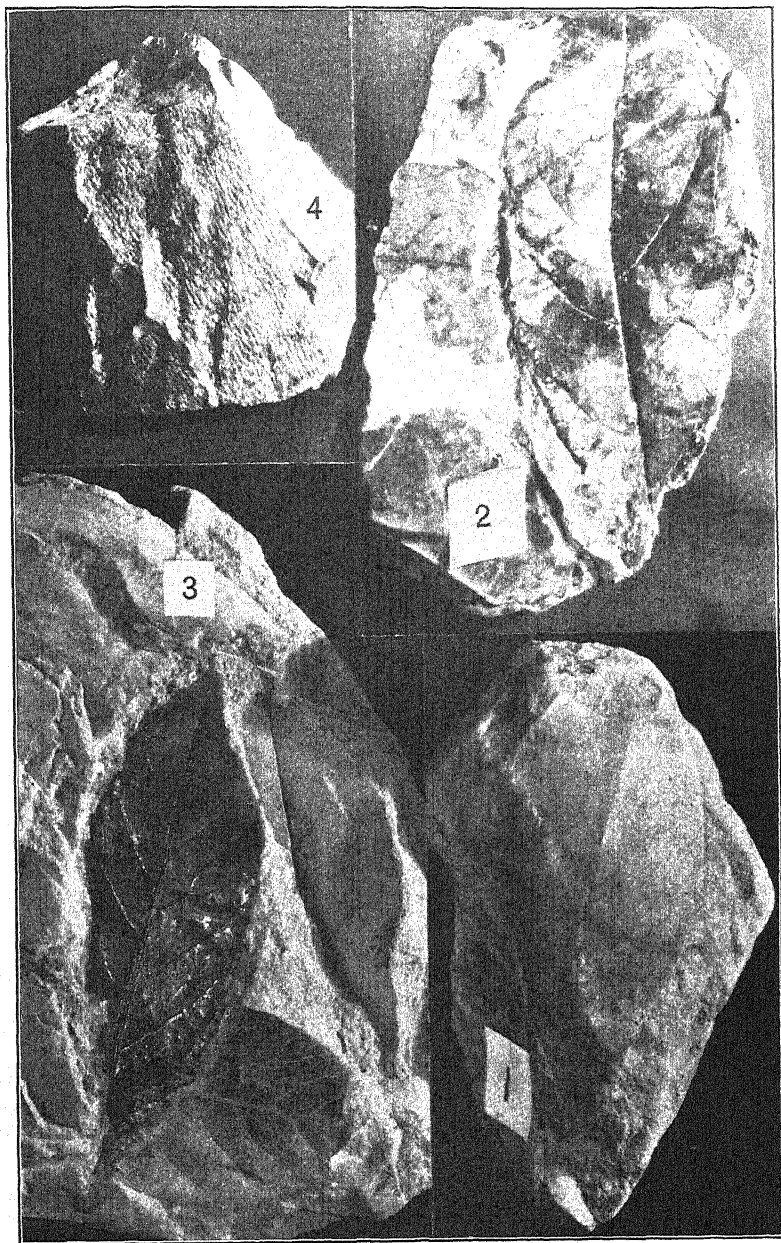
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Explanation of plate 16

- FIG. 1. *PRUNUS DEMISSA* Walp., leaf.  
FIG. 2. *PERSEA ALPIGENA* Spreng., leaf.  
FIG. 3. *FRAXINUS OREGONA* Nutt., leaf.  
FIG. 4. *PLATANUS RACEMOSA* Nutt., bark.  
All of the photographs are natural size.





SCOTT: TESLA REGION



## Changes in plastids in variegated plants<sup>1</sup>

ILLO HEIN

(WITH PLATE 17)

If on the margins of the chlorotic areas of variegated leaves there is a sharply marked transition zone between cells with normal green plastids and those with no plastids or with colorless plastids, it would seem that there are two types of cells present, whose characters may be transmitted independently in heredity. If, on the other hand, the transition from colorless cells to those with normal green plastids is gradual, it suggests the origin of the modified area by the diffusion outward from a center of some toxic substance.

Baur (2) believes that there are essentially two types of variegation: a very common non-infectious type which is more or less transmissible through seed, such as occurs in *Pelargonium zonale*; and a rarer type which is not transmissible through seed and is decidedly infectious, such as occurs in *Abutilon Thompsoni*. Baur's work on the chimera has been well reviewed by Küster (13) and by Noack (18), and on infectious types by Shull (20) and by Clinton (7). As a result of numerous experiments with infectious chlorosis in species of *Abutilon* and other members of the mallow family, Baur has concluded that it is caused by a chlorophyll destroying toxin which is produced in the infected leaves.

That variegated abutilons could be produced only by grafting has been known to horticulturists for over 200 years, and the method was described by Morren (17) and by Lindemuth (15). Morren demonstrated that the variegated abutilons could not be propagated by seeds.

For the non-infectious type of chlorosis in *Pelargonium*, Baur (4, 5) finds that the plastids in the white cells uniformly contain little or no chlorophyll and are smaller than the plastids in the green cells, but he does not state whether or not there is an area of gradation between the two types. The white cells arise from pre-existing white cells, and green cells from pre-existing green

<sup>1</sup> Contributions from the Department of Botany of Columbia University, no. 346.

cells, the two kinds arranged in sharply defined layers or sectors, these producing the so-called chimeras. The various arrangements of the tissues in these types have been discussed by Baur (5) Noack (18) Küster (13) Chittenden (6) Stout (20) Correns (8) and Bateson (1).

As a possible working hypothesis for the origin of the chimeral type of variegation, Baur (4) suggests the possibility that a fertilized egg which originated from the union of a 'white' sex cell and a 'green' sex cell would have two kinds of plastids, green and white, and the vegetative segregation of these plastids in successive cell divisions might result in various distributions upon which would depend the appearance of the leaves.

There may be pure green, pure white, or sectorial chimeral leaves and sprouts. In all these, however, the transition from green to white should be abrupt. Noack (18, 19) points out that the chimeral, the pure white, or the pure green sprouts could not arise by direct cytoplasmic inheritance and the vegetative segregation of the two kinds of plastids, because of the relatively small number of plastids that can possibly be contributed by the male cell, and the very large number of plastids to be expected in the egg cell. In such crosses as he claims the hereditary factors must be found in the nucleus.

Data supporting the theory of the transmission of variegation by chromosomal genes have been reported by Chittenden (6). Anatomical studies of certain varieties of *Pelargonium zonale*—Freak of Nature, Caroline Schmidt, and Baur's B—and of *Hydrangea hortensis* show sharply differentiated white and green tissues; in the former there are no plastids and the change from white cells to green cells is abrupt. For these the assumption of distinctive factors for white and green may seem plausible. However in a variety of *Pelargonium*, Happy Thought, whose leaves are greenish yellow in the center surrounded by a green border, the sections do not show a sharp boundary between the yellow and green cells, but there is a region several cells thick in which gradations from pale yellow to green occur. The plastids are difficult to see in the pale areas and become greener in the area of transition. Chittenden states that this is not a periclinal chimera with white central tissue surrounded by peripheral green cells, but holds that, "Presumably this yellow tissue is due to heterozygosis with one or more bleaching factors, the recom-

binations of which with each other and with the normal green" give the results. It does not seem clear from Chittenden's account how factors for bleaching and factors for green give the intermediate stage in the marginal cells. The assumption of some substance diffusing radially from cell to cell and gradually bleaching the plastids seems simpler for such cases.

The condition of the plastids in the modified areas of many variegated plants has been described by Zimmermann (23). He finds that the plastids show gradual changes in color and size from the margin to the center of the modified areas in *Farfugium grande* and in *Achyranthes Verschaffeltii*. Küster (12) has studied the plastids in the discolored areas of *Pelargonium zonale*, *P. peltatum*, *Spiraea Bumalda*, and *Ligustrum ovalifolium*, and states that they are incompletely developed or have become bleached and degenerate, apparently through agencies within the cells. Stout (21) states for *Coleus*, "Plastids are present in both green and yellow cells but in yellow cells they are fewer in number, smaller in size and somewhat distorted in shape . . . in extreme cases of yellow development nearly all the cells fail in the production of chlorophyll."

Woods (22) holds that in such cases of variegation the chlorophyll is destroyed by oxidizing enzymes, oxidases and peroxidases, which are normally present in all green plants and under certain conditions may be produced in abnormally large quantities. Woods believes that tobacco mosaic is due to an excess of oxidizing enzymes rather than a *contagium vivum fluidum* as described by Beijerinck.

Very suggestive work has been done by Liesegang (14) Küster (11) and Gebhardt (9), who point out the similarity between the Liesegang patterns and the white and green markings in certain variegated leaves. Küster filled a glass tube with 1/10 per cent gelatine chromate and inserted one end into an 80 per cent solution of silver nitrate. A series of alternate horizontal dark and light zones were formed, which, though the distances between the rings are very short, suggest the green and white zones in such plants as *Scirpus zebrinus*. Instead of simply depositing a drop of the nitrate in one place, various lines and figures may be drawn, and thus many leaf patterns may be imitated. Gebhardt has imitated butterfly wing patterns by arranging the solutions so that the resulting concentric zones formed similar figures.

I have studied conditions of the plastids in the cells at the margins of the chlorotic areas in a number of variegated plants which are classed by Baur as non-infectious, inheritable types. As it seemed desirable to observe the cells in as near their natural condition as possible, sections from 7 to 10 microns in thickness were cut with the aid of the freezing microtome and mounted in water. These sections were cut so that they included both normal green and abnormal white or yellow cells, and of course the more critical marginal cells lying between these two extremes.

*Dieffenbachia Seguine* Schott has large thick green leaves that are profusely blotched with irregularly shaped, white to yellow spots of various sizes. Excellent colored illustrations of the leaves may be found in Lowe's "Beautiful Leaved Plants." In the pale areas of the leaves there is a gradual decrease in the size of the chloroplasts as the chlorotic areas are approached. Figure 1 shows a typical cell from the normal green tissue; the chloroplasts are deep green, plump, and average from 8 to 10 microns in diameter. In figure 2 is shown a cell which was selected from those exhibiting the first decided changes from the normal. The smallest chloroplast in this cell measured 3 microns while the average size was 6 microns in length. A few of the plastids were apparently like the normal ones in healthy cells. Figure 3 represents a cell which was selected from the marginal cells which were about midway between the white and green tissues, and which showed a still greater decrease in size, color, and in the number of the chloroplasts, which were also considerably distorted. Figure 4 represents a cell that is almost colorless, showing only a few highly refractive pale green amorphous masses, which appear to be disorganized chloroplasts. At the base of the cell may be seen three pale green bodies which still retain a resemblance to chloroplasts, but they are very small and lack the amount of chlorophyll found in healthy plastids. Figure 5 shows a section through the critical zone. At the extreme right may be seen healthy cells with large, plump, normal plastids. Towards the left we see a gradual decrease in number, size, and color of the plastids until cells showing no green are reached. In these latter there is no evidence of any chlorophyll bearing bodies. The nuclei so far as I am able to determine are like any other resting nuclei and show no modifications in size, shape or general appearance.

Leaves of *Dracaena Godseffiana* Sander have irregularly distributed yellow to white rounded spots which vary in size. Sections through a yellow spot show that there are no chloroplasts in the abnormal tissue. The marginal cells from such a spot show from one to several plastids, which are irregular in shape, somewhat smaller, and contain less chlorophyll than the normal ones in the green tissue (FIG. 7a). Here and there in certain marginal cells chloroplasts can be found that show no visible differences from the normal (FIG. 7b). The general tendency of the plastids in the marginal cells is to be on that side of the cell which is nearest the normal green tissue, though they frequently may be found on the opposite side. The transition from normal green to colorless in this case is more abrupt than in *Dieffenbachia*, but is not absolutely sharp and definite, since the marginal cells show both normal and abnormal chloroplasts.

The rounded yellow spots of the leaf of *Ligularia Kaempferi* Sieb. & Zucc. (*Farfugium grande* Lindl.) show a gradual diminution in the amount of green color from the solid green cells at the border to the almost colorless central tissue. The areas consist visibly of more or less definite concentric zones, which range from normal green through various tones of green to yellow, and in the larger spots to white. This pattern is very suggestive of the Liesegang rings referred to above. The solid green parts of the leaf, on examination, present a characteristic marbled appearance due to variations in color. At first sight this would seem to be due to differences in the thickness of the leaf, since most of the deeper shades of green are near the veins, but closer examination reveals variation in color of the vein islets, and sections show that the leaf is actually of uniform thickness in the marbled areas. Sections of fresh material show no perceptible differences in the number, size and form of the chloroplasts in the darker and lighter parts of the leaf, and the cells are in general apparently normal. A slight variation in the intensity of the green color of the plastids may be detected, and it is to this that the marbled appearance is due. On the very margin of the modified spot the chloroplasts appear normal except in color, which is a little paler than the normal green, and four or five cells farther away from the margin there is a gradual falling off in the intensity of color until a delicate tint of green is reached, accompanied by a slight irregularity in form and size

of the plastid. From this point on the plastids become irregular in form and become more and more disorganized until they appear in the cells of the white tissue as colorless, amorphous masses. Figure 6 shows a relatively short transition zone between the white and green. In the middle of the spot there are no visible chloroplasts.

The chloroplasts in the pale zones of the sedge *Scirpus zebrinus* show degenerative changes similar to those described for *Diefenbachia* and *Dracaena*. There are no plastids in the middle of the colorless areas, and a few abnormal ones in the marginal cells.

Other variegated leaves studied, from species of *Croton*, *Abutilon*, and *Plantago lanceolata*, showed practically the same gradual degeneration of the plastids on the margins of the discolored areas as in the above described plants.

In all these cases it would seem that the spotting must be caused by a chlorophyll destroying agent which spreads radially and disorganizes the plastids as it penetrates from cell to cell. The possible nature and origin of such a virus or toxic agent is generally admitted to be as yet quite obscure.

The variegated form of *Oplismenus compositus* Beauv. (*Panicum variegatum* Hort.) shows much more marked differences in the cells of the green and white areas. In the white areas the mesophyll is almost completely atrophied, and the epidermal cells are hypertrophied to the extent that the thickness of the leaf in this region, even if there is no mesophyll tissue, is equal to the thickness of the green part of the leaf, in which there are regularly two layers of mesophyll cells. The two layers of the normal mesophyll consist of a palisade layer which is not much differentiated, and one other mesophyll layer. The chloroplasts are large, deep green and plump. The average thickness of the green portion of the leaves is about 100 microns in the lamina between the veins. The upper epidermal cells average 32 microns in thickness, the lower epidermal cells are somewhat thicker, while the two green mesophyll layers together have an average thickness of 32 microns (FIG. 9). In the colorless areas the thickness of the leaf is about the same as that of the normal green bands, but, as stated, there is practically no chlorophyll bearing mesophyll, the space between the epidermal layers is reduced to negligible dimensions, and in many places the epidermal layers are so hypertrophied as to completely fill the



space between them. The lower epidermal cells measure from 25 to 30 microns in the white areas (FIG. 8). Where the upper and lower epidermal layers do not touch there are sometimes very small cells to be seen, which may contain a plastid or two, or there may be simply large or small intercellular spaces. Sections of the leaves of the green form of this grass show a structure which is identical with the green regions of the variegated form. It is of great interest in this case to know whether the mesophyll is hypoplastic, there being an inhibition of cell division, or whether the mesophyll cells are formed and then atrophy. I am continuing my study of this form and certain others.

This work has been done under the direction of Professor R. A. Harper, and it is a pleasure to record my indebtedness for his stimulating suggestions during its progress, and for his valuable criticisms in the preparation of this paper.

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### Explanation of plate 17

FIG. 1. DIEFFENBACHIA SEGUINE: an apparently normal cell from the green portion of the leaf.

FIG. 2. Cell from extreme margin of a white spot. Plastids are smaller and have fallen off slightly in color.

FIG. 3. Cell from marginal area and nearer the white than the foregoing. Plastids are still paler and tend to be slightly distorted.

FIG. 4. Cell showing only three very pale irregular chloroplasts at the bottom, and two disorganized masses of pale green material, evidently degenerate chloroplasts, at *a* and *b*.

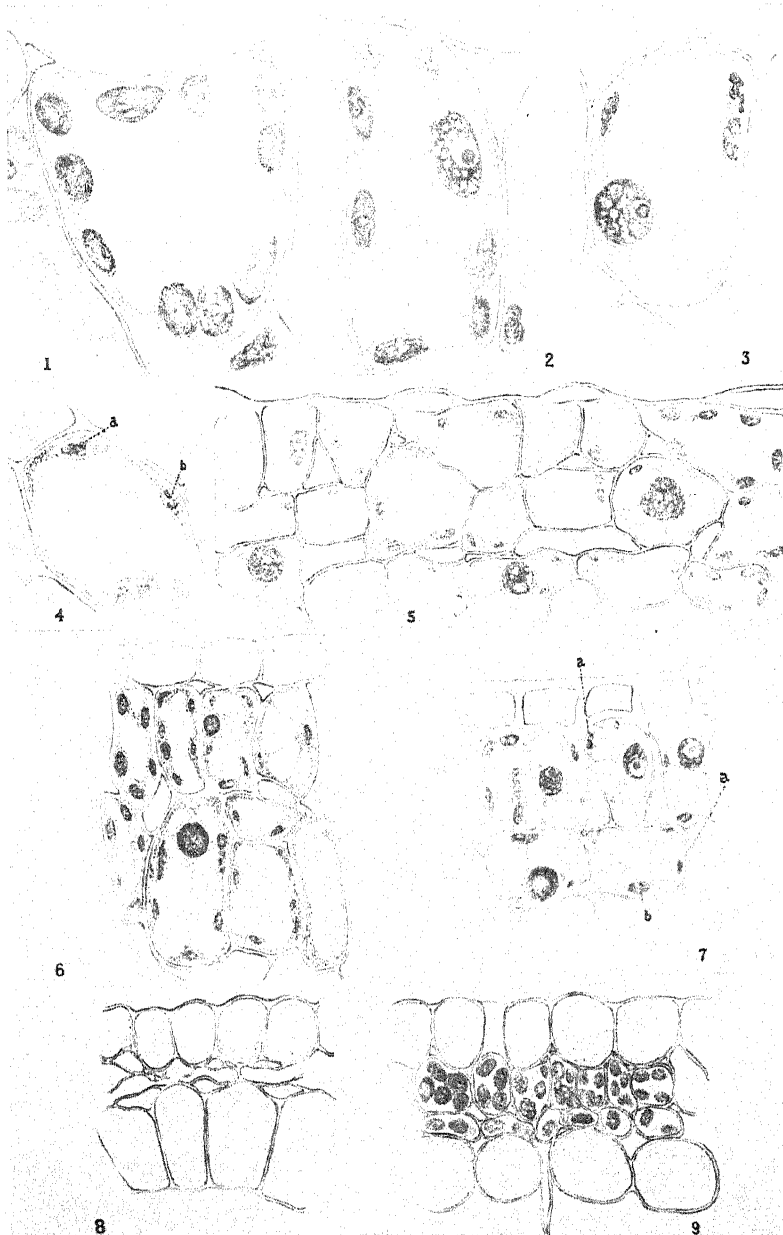
FIG. 5. Section through the marginal area showing apparently normal green cells at the extreme right. To the left the cells show successive stages in the degeneration of the plastids.

FIG. 6. LIGULARIA KAEMPFERI (*Farfugium grande*): cells from marginal area of yellow spot. Upper left cell normal; other cells show various stages of degenerating chloroplasts.

FIG. 7. DRACAENA GODSEFFIANA: upper left is normal cell. At *a* are pale irregular plastids. At *b* is an apparently normal plastid in a cell showing degenerated plastids.

FIG. 8. OPLISMENUS COMPOSITUS (*Panicum variegatum*): cells from white area, showing hypertrophied epidermal cells and absence of mesophyll.

FIG. 9. Section through the green area; mesophyll present and plastids green and plump. FIGS. 1-4,  $\times$  about 770; FIGS. 5, 6, 7,  $\times$  about 365; FIGS. 8, 9,  $\times$  400.



HEIN: PLASTIDS IN VARIEGATED PLANTS



# INDEX TO AMERICAN BOTANICAL LITERATURE

## 1904-1925

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Reviews, and papers that relate exclusively to forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included, and no attempt is made to index the literature of bacteriology. An occasional exception is made in favor of some paper appearing in an American periodical which is devoted wholly to botany. Reprints are not mentioned unless they differ from the original in some important particular. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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Respiration of potato tubers after injury

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The suggestion for this study of the respiration of injured potato tubers came from some results which were obtained in a previous investigation by the writer (11). In order to obtain sufficient material to make a chemical study of the changes which occurred in the regenerating layer induced by cutting tubers, Mr. N. L. Walbridge, instructor in physics in the University of Vermont, who was assisting the writer on the chemical determinations, and the writer cut in two about thirty tubers and kept them in a cool cellar covered with damp paper. At the end of six days a thin layer (about 1-2 mm. in thickness) was sliced off each cut surface and a similar layer for a check. The tubers were then replaced and allowed to regenerate a new layer, and the slicing operation was repeated in five or six days. Altogether, four such cuttings were made, and the cut slices dried and analyzed separately by Mr. Walbridge. The curious fact came out that the percentage of glucose and of fat increased with each successive cut, while it remained the same in the check samples. It seemed that we had here an example of a summation of stimuli, resulting, each time the stimulus was repeated, in an increased response in the form of a chemical deposit for the healing of the wound. The glucose is used as a source of carbohydrate material for respiration, so the question at once arose:—"Will the respiration show a similar rise with each stimulus?"

Before discussing results obtained, it will be best to call attention to the morphological changes, and to examine some of the literature that has already been published on the subject of respiration in injured plants.

The healing of the cut surface of a potato tuber takes place

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in two phases, provided a fairly warm temperature, dampness, and oxygen are available. A temporary stoppage layer is put in at once during the first day or two, and a new cork cambium regenerates and gives rise to a new skin in the third, fourth and fifth days. The first process consists mostly in the impregnation of the walls of the outer two or three layers of cells with suberin or similar fat-like compounds. These walls are less permeable to water so that the tuber loses less water, and they also exclude bacterial or fungal invaders. The second phase of the healing takes the form of the growth of a new cork cambium and cork layer. A layer of cells, five or six rows deep, free themselves from storage starch, acquire more protein in the nuclei and develop into a cork cambium. The new layer is about eight to ten cells in thickness, and is apparently as efficient in checking transpiration and respiration as the corky covering of the remaining parts of the tuber. The cell walls are suberised and the surface is further protected by three to five layers of cells on the exterior. These cells lose their protein contents and nuclei, but their suberised walls must materially assist in reducing the loss of water and carbon dioxide. The further fact was brought out by the writer that the regenerating layer is filled with glucose, which can be demonstrated micro-chemically with Fehling's solution, the region adjacent to the old skin being especially rich in it. This two-phase healing of wounds in plants has been shown by Priestley and Woffenden (15) to be a common occurrence, not only in the potato, but in a number of other species studied by them.

The physiological aspects of such plant injury have been studied by a number of investigators, by several with reference to the amount of carbon dioxide evolved at various stages in the process, while others have followed the chemical changes induced in injured tubers and bulbs.

Boehm (2) was apparently the first investigator to call attention to the abnormal production of  $\text{CO}_2$  in potatoes injured by cutting. He noted that this increase reached its maximum on the second day and then began to drop.

Boehm's later paper (3) laid emphasis on the traumatic effect of the cutting of the tubers on the tissues themselves, regardless of the presence or absence of oxygen. But this theory had already been overthrown by the investigations of



Stich (18) who covered the cut surfaces of the potatoes with neutral gelatin and fastened them together so tightly as to exclude all oxygen, and who found that no evolution of carbon dioxide resulted. The contact of the cut surface with the air was absolutely necessary to induce the reactions which produce the carbon dioxide. Attention should be called to the fact that he washed off the cut surfaces of the tubers with water, before covering them with a 2 mm. layer of 30 per cent gelatin. As will be noted later, this procedure may account partially for the results.

Richards' paper (16) on the respiration of wounded plants is the fullest account that we have of the production of  $\text{CO}_2$  after this type of stimulation. Potatoes, carrots, beets, young bean and melon seedlings and various sorts of twigs and leaves were used in the experimentation. The general method was to determine not only the  $\text{CO}_2$  produced, but also the oxygen consumed, by means of a modified form of the Pettenkofer apparatus. His data demonstrate that the increased respiration is at its maximum about two days after injury and that the absorption of oxygen is in excess of the amount of  $\text{CO}_2$  produced. This abnormal use of oxygen and excretion of  $\text{CO}_2$  he ascribes to an attempt on the part of the injured plant to free itself from an unnatural condition, that is to say, a cut surface open to the air, and that in the healing process all the vital activities are accelerated. When the open surface is blocked up by new tissue, the protoplasmic process again drops back to normal. Artificially blocking the open wound by applying wet clay would produce the same decrease in the amount of  $\text{CO}_2$  formed and oxygen used.

Friedrich's contribution (6) to the study of cut surfaces is on the chemical changes which are induced. Ordinary chemical methods of analysis were used on injured onions, potatoes, apples and quinces. The potatoes that were injured increased in total nitrogen and in acidity, and decreased in carbohydrates. Microchemical tests showed a marked deposit of reducing sugars along the cut surfaces. The reducing sugar is used up in the respiration induced by the healing. The organic acids formed are the intermediate products of respiration, a partial destruction of the glucose into  $\text{CO}_2$ . The glucose arises according to Friedrich's view from the destruction of the smaller starch grains,

but he gives no evidence of any observations that support this assumption.

Palladin (13) has attempted to distinguish three types of respiration:—(1) the  $\text{CO}_2$  has its origin in the nucleic acid; (2) the  $\text{CO}_2$  is a product of stimulation; (3) the formation of  $\text{CO}_2$  is due to oxidases. The  $\text{CO}_2$  produced in injuries would at first be, therefore, of the second type almost entirely, while later it might come partly from the destruction of the nucleic acid. The  $\text{CO}_2$  of stimulation is the result of the actual excretion of this gas by the protoplasm itself without the intervention of enzymes or oxidases. Quinine stimulates the protoplasm, as does etherization, and an increase in  $\text{CO}_2$  formation is the result.

Kovchoff (9, 10) confirmed on onion bulbs the chemical work of Hettlinger (8) and Zaleski (21), demonstrating the increase in protein in injured tissue, and he discovered further that the greater part of this increase was in the nucleoproteids that are not digestible in pepsin. This accumulation of nitrogenous compounds occurred only in the presence of oxygen, since injured bulbs placed in an atmosphere of hydrogen showed no protein increase. The non-digestible proteins are formed slowly during the first two days, but very rapidly during the following three or four days. Unfortunately his tests did not extend more than six days.

Kovchoff (10) later attempted to demonstrate by comparison of the phosphorus and nitrogen ratios that the formation of these indigestible nucleoproteins was not a form of starvation. The ratio of phosphorus to nitrogen remained the same in injured or uninjured tissue.

Smirnoff's work (17) is interesting in that he determined the amount of intramolecular respiration in injured onion bulbs by placing them in an atmosphere of hydrogen. The respiration which had been measured by Richards, Stich, and others was made up of two components; intramolecular and wound. The latter component only shows in the presence of oxygen, so, when the bulbs were placed in the atmosphere of hydrogen, only the intramolecular respiration remained. Smirnoff shows that this respiration was not increased by the wound stimuli.

The classic paper of Müller-Thurgau (12) on the accumulation of sugar in tubers stored at low temperatures is often

quoted, and may offer some side lights on the problem of respiration and sugar formation in tubers that have been kept at low temperatures. As is well known, potatoes kept at zero Centigrade, or at nearly that temperature, become sweet to the taste from an accumulation of sugar. This investigator concludes from a long series of trials with tubers kept at various low temperatures with a subsequent chemical analysis of them, that the accumulation of sugar is a normal process associated with the rest period of the plants. The enzymes which convert the starch into sugar continue to act even at low temperatures, while respiration is reduced to a minimum. The consequence is an increase in the sugar content. Such sweet tubers, when brought into a warm laboratory, respire more, and germinate quicker, than those kept at higher temperatures. The sugar that has been accumulated is available either for the formation of carbon dioxide, or to feed the young sprouts. The particular contribution that he suggests is that the diastatic enzyme continues to act in the cold, while the respiration hormones, if there are such bodies, are inactive.

Butler (5) more recently has confirmed these conclusions, and has further shown that potato varieties differ in the rapidity with which they accumulate sugar, that the sugar is located mostly at the eyes and apparently very little of it is translocated. He is inclined to associate the accumulation of sugars just at the time sprouts are formed with other metabolic agents.

Votchak's results (20, according to Palladin's *Plant Physiology*) show the presence of a larger percentage of solanin in the injured portions of the potato tuber. Palladin's suggestion that the solanin may serve as a stimulus to respiration is a very plausible one, and may explain also the larger percentage of glucose in the injured portions next to the old uninjured skin, since the solanin is most abundant there and in the neighborhood of the potato eyes. In the ordinary starch layers there is only .002 per cent, while in the skin there is 0.7 per cent (Wehmer, *Die Pflanzenstoffe*, p. 681). On the other hand, the increase in solanin content may be only another expression of the contact with oxygen, the two processes—increase of solanin and rise of respiration—being coincident, but not dependent the one on the other.

The increased respiration after injury, while it is in part

induced by the healing process, may be regarded as in the main an irritable response of the protoplasm to the stimulus of the contact of tuber cells with the oxygen of the air. These cells, deeply imbedded in the tuber tissue, are under ordinary conditions protected by a cork layer from such a contact, and are only brought to the outer surface by violent means such as the cutting or breaking of the tuber. As Verworn (19) says, "Every living system possesses, as we know, a peculiar and characteristic manner of reacting to stimulation. The muscle responds with a contraction, the salivary cells with production of saliva, the luminous cells with the emission of light." In the cut or injured tuber cells, the response is in the production of carbon dioxide and the deposition of sugar in the injured part. The problems of irritability have been studied mostly from animal protoplasm. A review of them in their general aspects may be found in the book of Verworn just quoted, especially applicable to the present study being the chapter on "The refractory period and fatigue." The refractory period lies between the successive stimuli. As the organism gradually recovers from a stimulus, it frees itself from the products that have been laid down in the cells as a result. These products may be regarded as the cause of asphyxiation. The presence of oxygen will disintegrate these products and in the course of a varying length of time, the effects of the stimulus and its products will disappear. As long as some of these products remain, the response will not be as great and we say that the cells are in a state of fatigue. Fatigue, therefore, is really only a form of asphyxiation, due to a lack of oxygen supply, or to an insufficient length of time having elapsed since the previous stimulus. If the stimuli come rapidly with very short intervals of rest, fatigue is induced with few stimuli, but if the stimuli come at long intervals, the decomposition products pass off into the surrounding air as carbon dioxide. It has been shown for example that a frog's leg stimulated until completely fatigued will not regain its irritability in an oxygen free medium, while it will as soon as it is placed in oxygen.

Bose (4), using electrical stimuli on *Mimosa*, found that if he distributed the stimuli so as to allow an interval of rest of fifteen minutes, the responses were all of the same height of curve, but that if only ten minutes intervened there appeared

a fatigue diminution. The objection may be raised to Bose's work that he, by using electrical stimuli, induced various chemical changes, ionizations, etc., in the irritated protoplasm, but it must be admitted that it is probable that that is exactly what occurs as the result of any stimulus, i. e. the response is chemical.

The literature cited above shows that we have accumulated considerable information on the respiration, chemical products, and cell structures involved in injured tubers and bulbs. The present investigation is an attempt to enlarge our knowledge of the effects of repeated cuttings on respiration and reducing sugar content, and of the influence which chemicals and external conditions will bring about in the amount of carbon dioxide produced.

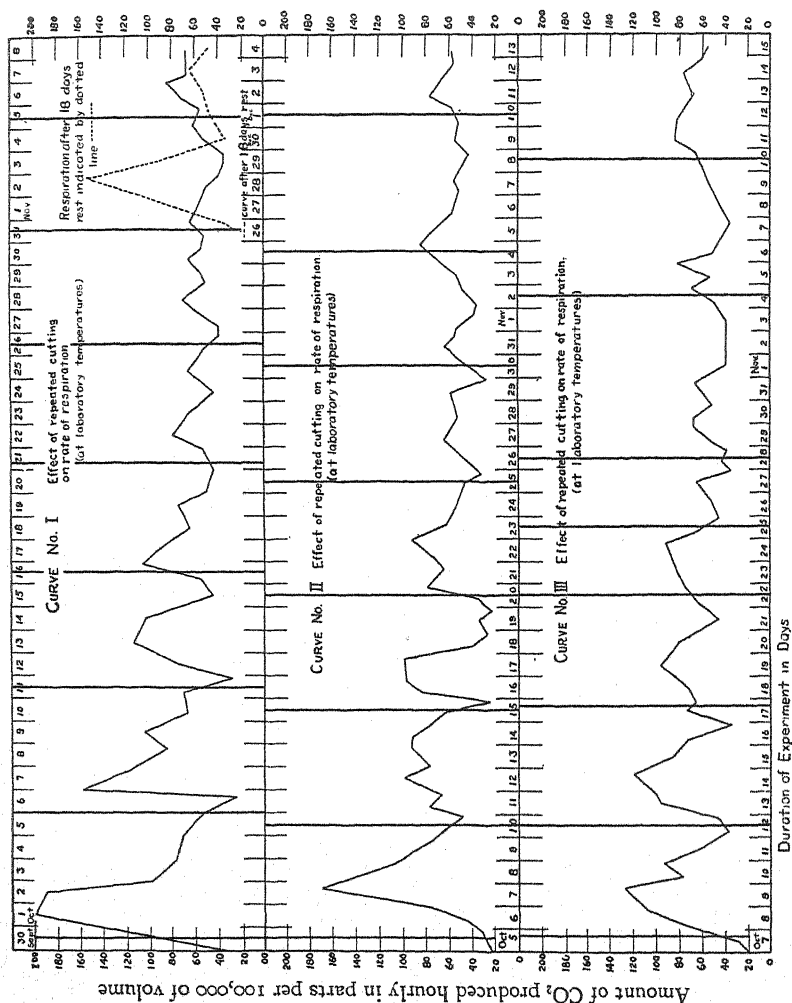
#### APPARATUS AND METHODS

Green Mountain tubers were brought into the laboratory at least a week before they were used for experimental purposes, except in one or two trials. The amount of carbon dioxide produced normally by the tubers was determined by placing them under a bell jar in a saturated atmosphere for a day before they were injured. The end of the tuber was then cut off, a thin slice taken for chemical tests, and the cut tubers placed under a bell jar on a piece of damp filter paper. The air in the bell jar was kept saturated, as indicated by drops of dew on the interior. The size of these jars varied from three to six liters.

The carbon dioxide determinations were usually made twice a day, early in the morning and late in the afternoon, although it was found necessary to do this only once a day on some of the experiments. The rate of formation of carbon dioxide per hour was used as a basis for comparison for each experiment.

The portable type of apparatus devised by Haldane for analyzing samples of air in coal mines was used for the carbon dioxide determinations. In the method for using this apparatus approximately a 10 cc. sample of the air is taken, accurately measured, the carbon dioxide absorbed by a strong alkaline solution, and the sample re-measured. The difference in the readings is the percentage of carbon dioxide. The most important precaution is to obtain a representative sample. This was

secured by thoroughly stirring the air in the bell jar with a celluloid fan attached to a glass rod, operated from the outside, before the 10 cc. samples were withdrawn. These samples were withdrawn through a pinchcock at the end of a long glass tube



extending down into the bell jar to a point just over the cut tubers. The sampling tube of the Haldane apparatus was filled at least three times by passing air contained in the connecting tubes over into the bell jar and filling them with the air from

the bell jar, before the readings were taken. Duplicate readings were taken in nearly all cases, and the two readings usually agreed within a few points. Samples taken at the bottom and at the top of the jar showed no measurable difference in  $\text{CO}_2$  percentage after the stirring. The method is accurate to one-tenth of one per cent.

The tubers under the bell jars were kept at laboratory temperature,  $21-22^\circ \text{C}$ ., but on certain Sundays the temperature fell at times to  $16^\circ \text{C}$ . This variation was unfortunate but could not be avoided. In the later experiments, a room which was heated by means of an electrically controlled electric radiator was used. The temperature in this room was maintained at about  $23^\circ \text{C}$ ., and never sank below  $19^\circ \text{C}$ .

The weight of tubers used varied in the various experiments, as did the size of the bell jars. The comparisons were made entirely on the rate of production of carbon dioxide per hour under a given bell jar, with a given lot of potato tubers cut in a certain way.

## RESULTS

1. *Effect of repeated cutting on the respiration.* The first set of experiments were conducted to ascertain the effect of repeated cutting on the production of the  $\text{CO}_2$  of respiration. Four or five large tubers of a weight approximating 450-500 grams were washed and placed under the bell jar for at least a day before the experiment. The normal rate of respiration of the uninjured tubers having been obtained, the end of the tuber was cut off to a point where the diameter was fairly constant. Usually half stem and half bud ends were cut off to equalize any possible difference in the respiration of the two ends. The cut tubers (not inclusive of the cut-off portions) were replaced under the bell jar, and readings taken again after an interval of 8-12 hours. Two readings a day were usually taken, one early in the morning at between seven and nine o'clock and the second later in the day at from four to six o'clock. This total production of  $\text{CO}_2$  was divided by the number of hours to get the rate per hour. The injured tubers were kept under the bell jar for 5-6 days, at the end of which time the respiration had again almost returned to normal. A thin slice, about 1-2 mm. thick, was again cut from each of the tubers and they were replaced under

the bell jar. These operations were repeated every 5-6 days until eight slices had been removed. The data can be best presented in the form of curves, where the height of the curve represents the amount of  $\text{CO}_2$  produced per hour in parts per hundred thousand by the injured tubers under one bell jar. CURVES I, II, III, IV, and V present this phase of the study.

The results of the first cutting are well known from the previous work of Boehm, Stich, Richards, and others, which has been mentioned. The curve of respiration rises after the first day, attains a maximum on the second or third day, and then gradually drops away. The maximum obtained seemed to vary with the various lots of tubers used, and also with their condition. Tubers brought in fresh from the out doors gave a much higher rate of respiration than those which had been kept in the laboratory for a week or two, since the curve of respiration was not only that of injury, but had the added effect, studied by Müller-Thurgau (12), of the rise of temperature induced by the change to the warm laboratory. The difference is illustrated in CURVE I from the respiration of tubers directly from the field, and CURVE II from tubers that had been a week in the laboratory. The second cutting did not produce as high a percentage of respiration in any case, the maximum of the second day being lower in every instance. The general form of the curve, however, was essentially the same.

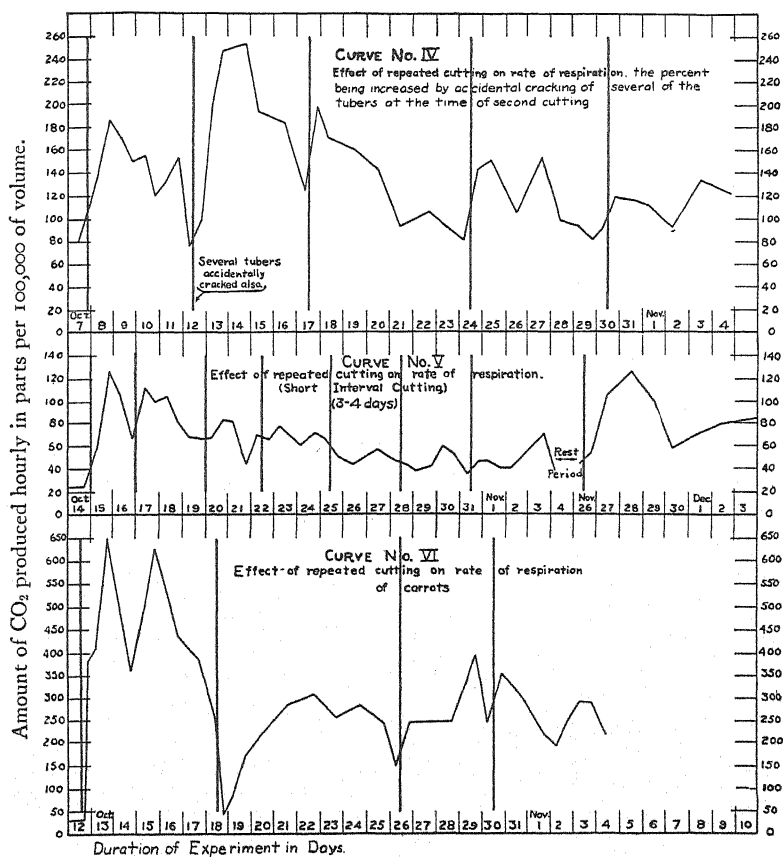
The third, fourth and succeeding cuttings produced less and less stimulative effect, until the reaction, as indicated in increased  $\text{CO}_2$  production, could hardly be noticed at all. The minor fluctuations which occurred between the later cuttings must be attributed to the variations in temperature to which the tubers were subjected in the laboratory. The stimulus of injury to the tubers is to be regarded as one which, if repeated four or five times, fails to produce a response so far as increase of respiration is concerned. Fatigue develops rapidly.

It was thought possible that the stimuli were applied at too long intervals; therefore the tubers whose respiration is recorded on CURVE V, were cut, not every 5-6 days, but every 2-3 days, in the hope of getting a cumulative rise, a stepping up of the reaction. The results, however, showed just the opposite. When the stimuli came at these intervals, the reaction disappeared with only two or three cuttings, and the curve of wound



respiration flattened out with only the irregularities due to variations in the laboratory temperature.

On the other hand, long intervals between the stimuli gave the plant an opportunity to recover from its fatigue, as can be seen in CURVE XIII, where the interval between the cuttings was 10-12 days. The later curves were almost as high as the



first one. The additional 5-6 days seemed to give the tuber a chance to recover its sensitiveness, at least as expressed in respiration, so that when the same stimulus was applied, the response was just about as vigorous as at first. This recovery from fatigue is further illustrated in the latter parts of CURVES I and V. The tubers used in obtaining these curves were kept

in the laboratory, after their susceptibility to injury stimuli had become exhausted, in the case of the first lot for 18 days, and in the case of the fifth lot for 22 days. The results obtained were surprising. The respiration response was almost as good as it had been in the first cutting. The fatigue seemed to have entirely disappeared.

2. *Increase of injury due to cracking.* CURVE IV presents the anomaly that the second cutting apparently produced a higher rate of respiration than the first cutting. The second curve was regular, apparently normal, and higher in rate than the first one, but an examination of the tubers at this time showed that, in addition to the second cut, two of them had small cracks on the angle formed by the cut surface and the old skin. It was remembered then that, in opening the bell jar and removing the tubers at the second cutting, two of them had slipped out of the writer's hands and dropped several feet to the cement floor. The cracks were the result. The injured surface is increased two-fold in each crack, since each side of the crack serves as a respiring surface. The interchange of gases would be almost as fast from the sides of a crack as it would from free areas.

This accidental observation suggested a trial of cracked potatoes for comparison with potatoes cut all the way across. By means of a very fine razor blade it was possible to cut the tubers just about half way through. Attempts at cracking were not successful, as it was impossible to determine the depth of the crack. As a check, tubers of the same size cut entirely across were placed under an adjoining bell jar of the same size. The cut surfaces were of the same area, counting the cracks as having double the surface of the ordinary cut. The respiration is indicated in CURVE XII, where it will be noted that the two curves are almost identical. At the close of the experiment the partly split tubers were broken apart to ascertain the size and depth of the injury, and it was found that the injury covered just half of the cross-section of the tubers.

3. *Respiration after bruising.* The respiration from the cracked tubers was so marked that other common forms of injury were investigated. Bruising was produced by a rubber hammer and by hitting the floor or table with the tubers. As far as possible, breaks in the skin were avoided, although a few

small ones did show after the rather rough treatment to which the tubers were subjected. The tubers were soft in many places. The respiration of the bruised tubers is shown in CURVE XVIII. The slight rise is doubtless due to the broken spots of the skin. The bruising seemed to have little effect provided the skin remained unbroken; contact with the air would seem to be necessary to produce this type of response to the injury stimulus.

4. *Respiration of scabby tubers.* The increase in respiration after infection with *Phytophthora infestans* has been observed by Boehm (3) and other investigators have confirmed this effect of fungus on host. Infection with the parasitic strains of *Actinomyces chromogenus* causes an enlargement and deepening of the lenticels of the tuber and affords an opening for the exchange of gases of the interior of the tuber with the gases of the air. The lenticular tissue, together with the corky layers formed as a result of the parasitic stimulus, is filled with large intercellular spaces that provide an easy channel for the interchange of gases. The respiration of equal weights of scabby and clean tubers kept under adjacent bell jars with moist filter papers is shown in CURVES VII and VIII. The rapid rise of the respiration of both clean and scabby was due to the fact that the tubers were brought into the warm laboratory from a cool storage cellar. After nine days it was noted that one of the scabby tubers had developed storage (*Fusarium*) rot, and it had to be removed. The curve dropped as a consequence. The scabby tubers, weight for weight, showed higher respiration than the clean ones. The presence of the tuber affected by dry rot cast some doubt on the observations so far as scab was concerned, so the experiment was repeated with a new lot, the curve of respiration being shown again to be consistently, although not markedly, higher in scabby tubers than it was in clean ones (CURVE VIII).

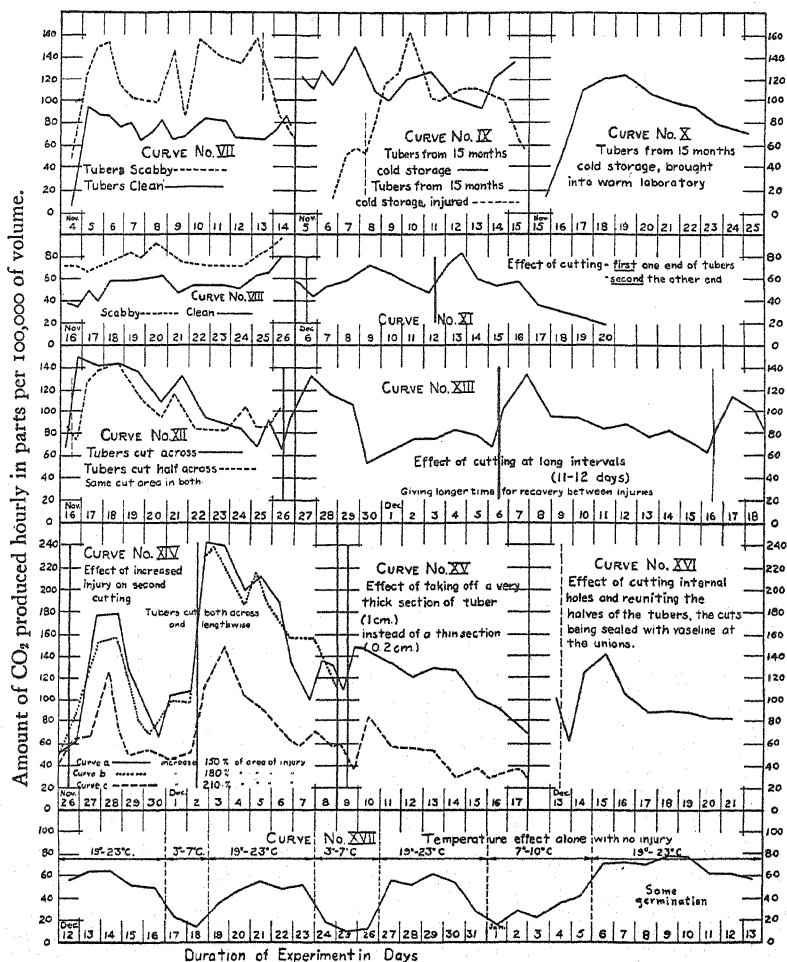
5. *Respiration and recovery from injury of old potatoes from cold storage.* A quantity of Green Mountain tubers had been held in cold storage since the preceding summer—about 15 months. The rate of respiration for such tubers rises rapidly when they are brought into a warm room, as shown in CURVE x, and seems to remain high, at least for some time. This curve only confirms the results of Müller-Thurgau (12) already referred to above.

In order to overcome this rise from a change of temperature, some tubers from storage were kept at laboratory temperature for about a week before being used for the injury experiments (CURVE IX). The respiration was taken for 3-5 days, and after it was found to be fairly constant, the ends were cut off one lot while the others were left as a check. The injured tubers produced the ordinary respiration curve of response, and in almost as marked a degree as those that were fresh from the field or had been harvested a month or two before. The long continued low temperature did not check the response to injury, although it did cause the tubers even when uninjured to maintain a high rate of respiration, the evolution of  $\text{CO}_2$  in this case, being, however, a normal process.

6. *The respiration curve in detail.* A careful examination of some of the curves of respiration after injury show two maxima, a high one after the first day and a secondary one after about three or four days, with a pronounced drop between. This comes out in CURVE I after the 2nd, 3rd, 4th and 5th cutting and in the curves formed after the tubers had rested for 18 days, in CURVE II after the 4th, 5th and 6th cutting, in CURVE III after the 1st cutting, in CURVE IV after the 1st and 4th cutting, and in CURVES XV and XVI after both the 1st and 2nd cutting. These respiration data were taken at laboratory temperatures which varied quite a number of degrees so that they could not be depended upon for fine details. In view of the fact that the healing process, as already explained, occurs in two phases, it was deemed advisable to try some injury experiments under more constant temperatures. For this purpose, a room with an electric radiator controlled by a thermostat was employed. The variation here was only between  $19-23^\circ \text{C}$ ., with the temperature held most of the time at  $22-23^\circ \text{C}$ .

A lot of five tubers weighing 930 grams was kept in this room for 5 days and then placed under a bell jar to determine the normal respiration per hour for 24 hours. At the end of this time each of the tubers was cut in two and half placed under each of two six liter bell jars. The curve of respiration is shown in CURVE XIX. Both lots showed a drop after the maximum obtained on the second day, with another rise in the latter part of the third day. After the third day the respiration gradually dropped away during the fourth to seventh days.

A second cutting made on the seventh day gave the peculiar effect of a diminution in the rate of respiration during the day on which the tubers were injured. The second day, the respiration was at its peak, after which a sag appeared in the amount obtained under one of the jars, with a rise again the next day.



It is apparent from the above that the shock of injury may check all normal respiration for a time, and that the first recovery phase, during which the cut surfaces are being suberised, produces the first maximum. The second maximum appears

just about as the cell divisions of the new cork cambium are most numerous, i. e. during the third to fourth days. The writer (11) has determined this fact by a series of trials made to secure as numerous cell divisions as possible in this regenerating layer for cytological study. The sag would appear at the time when the cork cambium is being differentiated. The gradual dropping away of the respiration curve is coincident with the divisions of the cork cambium—at the end of five to seven days, the new cork layers are about completed and the respiration is again reduced to about that of the normal skin.

7. *Respiration after increase of injury.* Richards (16) tried the effect of increase of injury on the rate of respiration in his experiment number 6. Tubers were halved, the respiration taken, and then each half cut into six pieces and the respiration again taken. This experiment is hardly comparable to the one devised by the writer to overcome the drop in the respiration curve after the second cutting, in that Richards did not wait until the wound respiration had attained its maximum and dropped back to normal. The method used in the present experiments was to cut another thin slice from the old wound, and then to halve some of the halves again. The original surfaces and the new wounded surfaces were measured by the planimeter.

Five tubers of a weight of 471 grams were cut across one end (part of them bud and part stem end) and the hourly respiration curve followed for seven days. The old wound was then cut again and one of the tubers cut at right angles, increasing the wounded surface to 150 per cent of its former area (CURVE XIV). Five other tubers of 485 grams weight were treated in the same manner, but two of the tubers were cut across at right angles, with an increase of 210 per cent of the injured surface (CURVE *b* of no. XIV). Five tubers, weighing 451 grams, were treated in a similar manner, but three of the tubers were cut across at right angles, with an increase of 210 per cent in wound area (CURVE *c*). All the bell jars used in these experiments were kept at a constant temperature approximating 22–23° C.

The curves of respiration from the second injury rose in all cases markedly higher than those from the original cuts. The amount of increase of injury did not seem to affect the propor-

tionate height, but the response was higher in all cases. It is to be pointed out that this experiment really does not increase the injury on the *old* surface, but opens up new tissues and cells to the action of the oxygen. It is doubtful if the injury stimulus extends more than a few millimeters below the cut; the chemical disturbance (as indicated by the presence of glucose) probably is a very good indication of its limits.

This suggestion that the wound stimulus was very superficial caused the writer to use the lot of tubers followed in CURVE *c* for deep injury. Instead of removing a slice 1-2 mm. thick, a piece 1 cm. in thickness was taken off. The resultant curve showed, however, the fatigue drop in exactly the same manner as when the slices removed were thinner. In the same way some of the tubers from which CURVES *a* and *b* had been obtained were combined, cut deep and replaced under a bell jar. The weight was approximately that of one of the original lots. CURVE *xv* shows that the reaction is similar to that shown from repeated cuttings of thin slices. The stimulus which produces the wound respiration and the fatigue must extend to some depth into the cell layers. The whole tuber in fact might be in a somewhat stimulated condition and show cell fatigue to repeated irritation. This possibility suggested the following experiment.

8. *Effect of cutting first one end and then the other of the tuber.* Four tubers weighing 380 grams were used after they had been in the laboratory for five days and their normal hourly respiration taken for one day. The ends of the tubers were then cut off, and the hourly respiration taken for six days. This particular lot of tubers did not show as marked a rise due to the injury as have some of the other lots, but there seems to be a great difference in the response among potatoes. At the end of six days the other ends of the tubers were cut off, 282 grams in weight being left, and the new cut surfaces approximating the old ones. The curve of respiration rose higher than from the first cutting. It is to be noted that the respiration from the first stimulus had disappeared before this second cutting. This experiment would indicate that the wound stimulus, while it extends to some depth in the tuber, does not affect all of it, and that, when the tubers were cut across at right angles to the old wound, part of this injury opened up a new surface which had been previously unaffected and was not markedly fatigued.

9. *Effect of internal injury on the rate of respiration.* The work of Bennett and Bartholomew (1) on the respiration of black heart tubers suggested the trial of some tubers cut internally and re-united.

Five large tubers with a weight of 498 grams were cut in two and a cavity about 3 cm. long, 2 cm. wide, and 2 cm. deep was excavated in each half. The amount cut out totalled 23 g. The normal respiration had been taken before injury. The two halves were re-united and bound together with twine, while a layer of vaseline was applied to the place where the cut surfaces came together. This vaseline was renewed several times while the respiration was being taken.

The curve produced (CURVE XVI) was almost exactly the same as that which resulted when the tubers were cut in two, a sharp rise the second and third day, with a gradual dropping back to the normal rate of respiration. The potato skin is apparently quite permeable through the lenticels to the carbon dioxide evolved in the interior cavities. Sections of the tissue lining this internal cavity showed the typical two forms of healing—a darkening of the outermost layer of cells, and a true cork cambium.

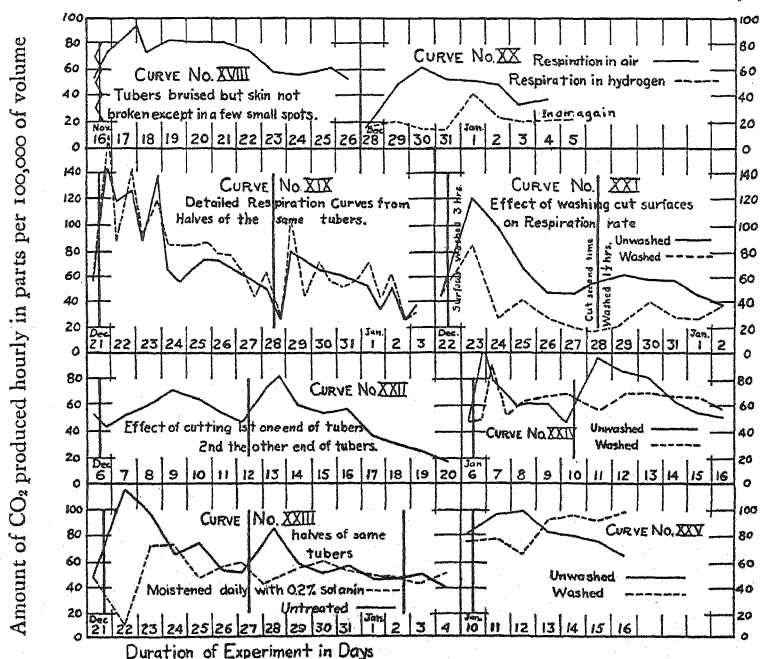
10. *Respiration after washing injured parts.* If, as has been suggested by some investigators, the process of respiration is induced by certain bodies known as respiration chromogens, these bodies being present in the plant cells and only becoming activated by the proper conditions, the query rose as to whether these bodies or substances could not be washed out of the surface cells after injury, with a resultant diminishing of the amount of respiration. The answer is in CURVES XXI, XXIV and XXV.

Five tubers weighing 606 grams, which had been in the laboratory a week, were kept under a bell jar for a day to determine the normal respiration. Each tuber was then cut into two equal halves. One lot of the halves was placed at once under a 4 liter bell jar on moist filter paper. The other halves were washed in a vessel in running water for three hours, and were then placed under another similar bell jar.

The respiration curve on the first lot (CURVE XXI) followed the usual course, with a maximum on the second day after injury and a gradual dropping off in the subsequent four or five days. On the other hand, the washed tubers, while they



showed some reaction on one day, dropped at once to the normal and retained this rate. It had been found in the preceding experiments that the loss in the height of the respiration curve could be compensated for by increasing the stimulus (CURVE XIV), the old surface being cut again, and one or more of the tubers cut at right angles. In this way the stimulus could be more than doubled. The first lot were cut only on the old surfaces, but the washed portions were cut not only on the old surface, but each tuber was again cut in two. The first lot



were placed at once under the bell jar while the second lot were washed for an hour and a half before they were replaced under a similar jar. The results with the first lot were a confirmation of the repeated cutting:—a marked lessening in height of the respiration curve, while the washed portion, in spite of the severity of the injury, gave practically no rise in the respiration rate.

This experiment was so interesting that it was thought advisable to try it again under somewhat different conditions.

After securing the normal rate of respiration of a lot of tubers (CURVE XXIV) the tubers were cut into approximately equal halves. One lot of the halves was placed under a bell jar on moistened paper at once, but the other lot was washed (after cutting) for about a half hour before being placed under a similar jar. While the respiration curve of the unwashed portions is higher than that of the washed, it was evident that the results were not nearly as striking as those which had been previously obtained, and the difference lay apparently in the length of time of washing. The same tubers, therefore, were cut again, but at right angles to the former cut. The washing this time was intended to be quite thorough, so it was continued for five hours before the tubers were removed to the bell jar. At the same time, another lot (CURVE XXV) was treated in a similar way. The loss in respiration of the washed lots was noticeable for the first three days, but after that time, the curves of washed and unwashed became roughly parallel. It will be recalled that the later healing of the cut surfaces consists in the formation of a new cork cambium, and that this does not develop before the third or fourth day after wounding. The layers of cells which bring about this regeneration lie at some distance from the surface, and would be little affected by any surface washing that might be given. The temporary healing which is characteristic of the first day or two, however, is superficial, and consists in the impregnation of the cell walls with various substances which are probably water soluble. According to Herklots (7), fatty acids are released from the tissue just below the cut, which suberize the cell walls of the superficial layers, provided the hydrogen ion concentration is maintained. The cut surfaces which have been washed are white in appearance, while the unwashed ones are light gray. Cross-sections made some days later, however, showed that a typical cork layer and cork cambium had been formed under these white and unaltered parenchyma cells on the exterior. This fact would speak against the theory that the formation of cork meristem is dependent on the stoppage of the exterior by an impermeable suberized zone.

The conclusion to be drawn from these experiments is that something is removed from these outer cells which is essential to the increase in respiration. This substance may be the fatty

acids which Herklots postulated, or it may be the respiration activators, or both. The contact of the oxygen of the air with this outermost row of cells is necessary to induce wound respiration, but if this substance or substances are washed out of the cells where it, or they, occur, the deeper placed cell rows are not stimulated, and no large amount of abnormal respiration occurs. The necessity of oxygen in this process is indicated in the following experiment, where the cut tubers were placed in an atmosphere of hydrogen.

11. *Respiration after injury, in an atmosphere of hydrogen.* Five tubers weighing 795 grams were used. They had been in the laboratory about a week. The normal respiration was determined for one day, and then each tuber was cut in two equal parts. One lot was placed under a bell jar with moist filter paper. The other lot was placed under a similar jar and hydrogen passed through for three hours, the air being excluded by a water seal.

The respiration curve was almost flat from the tubers kept in the atmosphere of hydrogen, while it followed the usual course under the bell jar containing air. These results are almost identical with those obtained by Smirnoff (17) from injured onion bulbs. The small amount of carbon dioxide evolved in the atmosphere of hydrogen must be produced anaërobically from the destruction of carbohydrates in the cells. Such a form of respiration is of course independent of the access of oxygen.

12. *Effect of solanin on the wound respiration.* Votchal (20) made the observation that solanin was always formed in unusual amounts in wounded tubers. An experiment was therefore conducted to ascertain the influence that a weak solution of this alkaloid would have on the rate of wound respiration. The normal rate of respiration was obtained for large tubers with a total weight of 860 grams. The tubers were then cut in two and placed under bell jars of the same size, one lot untreated, and the other moistened with a 0.2 per cent suspension of solanin in water. The respiration curve for the two lots of halves is shown in CURVE XXIII. It will be noted that the immediate effect was to check the respiration, but that, later, its effect seemed to be exhausted, and the respiration of the two lots more nearly paralleled each other. The effect was

noticeable in the early phase of the healing, when the cells involved were on the surface, but when the deeper placed cork cambium came to be formed, the influence of the solanin, being superficial, could not make itself felt, and the respiration at this later stage was just about the same as on untreated surfaces. A second cutting of the same tubers was made, with similar treatments and in general the same results.

The problem of the effect of the solanin is entirely unsolved. It may be the chemical product which lessens the wound respiration as it increases in concentration, or it may be only one of the accompaniments of the injury to the cells. The fact that it seems to check respiration experimentally would suggest the possibility that, in part, its function is to reduce the respiration rate. No experimental method suggests itself of impregnating the deeper layers of cells with a solanin solution such as would occur if it were produced naturally in a wounded surface. As long, however, as it comes in contact with the actively respiring cells of the outermost layer, it lessens the evolution of carbon dioxide.

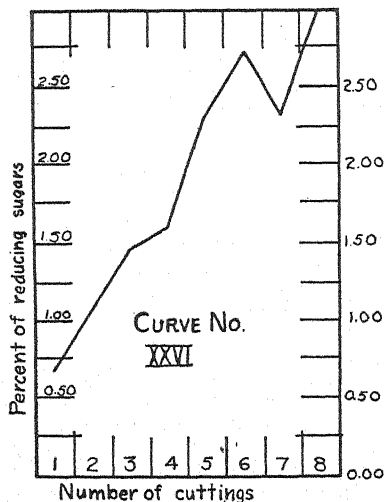
13. *Effect of alternating warmth and cold on the height of the respiration curve.* The very irregular curve produced during the periods of repeated cuttings suggested the possibility that, even if a tuber is uninjured, the curve might rise higher than normal after a few days in a very cold room. CURVE XVII presents the respiration during alternating periods of cold and warmth. The tubers were acclimated for about a week in the warm laboratory with electrically controlled temperatures, and then the respiration under a bell jar in a saturated atmosphere was taken for a few days; they were then placed in a very cool room for several days, the respiration being taken every day, before being returned to the warm controlled temperature room. This alternation was repeated several times, as the curve shows. The height of the respiration curve in the warm room, however, did not change much after such treatments, until just at the last rise, when several of the tubers began to sprout, and the experiment was discontinued. The results would seem to indicate that the storage of reducing sugars which Müller-Thurgau (12) found in tubers kept for some time at low temperatures did not occur in sufficient amounts to affect respiration, if the cold storage period was a very short one and the temperatures did not approach zero Centigrade.

## EXAMINATION OF THE INJURED TUBERS FOR REDUCING SUGARS

Since the inception of this investigation was due to the accidental discovery that the percentage of glucose increased in the injured portions of potato tubers, especially if the injury was repeated, the removed slices of the tubers used in the present investigation were dried and analyzed for glucose. If the slices removed happened to be a little thick, the per cent of glucose would drop, since it is nearly all in solution in the outer layers. On the other hand, very thin slices might barely remove all the glucose-containing layer, but none of the underlying tissue, and the apparent percentage would rise. The size of the samples varied, but after they were dried they were usually so small that there was no opportunity for running duplicate determinations. The figures that are given are derived from a single analysis, usually of all the material that was available. The methods recommended as official by the Association of Agricultural Chemists were carefully followed, but, as will be seen, the small amount of material and the variability of that

material gave results that were very widely divergent. The analyses are shown in TABLE I.

Very little comment can be made on the results as shown in percentages of glucose. In general, there is a rise in reducing sugars as a result of the injuries, Nos. II and III having a fairly regular increment after each cutting. By combining and averaging the results from the various cuttings, some of the deviations are smoothed out and a fairly regular curve can be obtained (CURVE XXVI).



The glucose (reducing sugars) represents material that can be easily worked over by the regenerating layer to form cellulose, or for oxidizing in the life processes, and it is evident that the plant supplies it in abundance when injured. If the injury is

TABLE I

*Showing in grams, green weight of portions removed, the dry weight, the weight analyzed, and the percentage of reducing sugar found*

No. of sample	Green weight	Dry weight	Weight used	Percent reducing sugars	No. of sample	Green weight	Dry weight	Weight used	Percent reducing sugars
I <sub>1</sub>	31.5	*	7.322	.35	IV <sub>5</sub>	18.5	4.0	3.849	2.23
I <sub>2</sub>	15.5	2.3	2.890	.67	IV <sub>6</sub>	21.8	7.7	3.318	5.07
I <sub>3</sub>	21.2	3.8	3.769	.58					
I <sub>4</sub>	22.0	3.8	3.760	1.18	V <sub>1</sub>	35.0	6.5	4.704	.07
I <sub>5</sub>	28.5	5.3	5.550	2.04	V <sub>2</sub>	—	4.0	3.558	.43
I <sub>6</sub>	27.5	5.5	5.450	1.96	V <sub>3</sub>	21.5	3.7	3.761	1.53
I <sub>7</sub>	21.7	4.5	4.026	1.04	V <sub>4</sub>	18.0	3.1	3.083	.41
I <sub>8</sub>	21.5	5.0	4.134	1.28	V <sub>5</sub>	14.5	2.2	2.517	2.84
I <sub>9</sub>	33.7	11.7	3.875	1.00	V <sub>6</sub>	18.5	3.5	3.186	.56
					V <sub>7</sub>	19.2	3.7	3.292	2.55
II <sub>1</sub>	—	*	5.722	.49					
II <sub>2</sub>	23.8	5.3	5.141	1.74	XIV <sub>1</sub>	41.0	12.0	4.994	1.43
II <sub>3</sub>	24.5	5.5	5.050	2.47	XIV <sub>2</sub>	18.5	6.0	2.761	3.00
II <sub>4</sub>	18.5	3.7	3.911	2.30	XIV <sub>3</sub>	28.0	6.0	5.132	4.00
II <sub>5</sub>	22.7	4.7	4.778	2.08	XIV <sub>4</sub>	31.0	6.6	4.111	1.76
II <sub>6</sub>	25.0	5.7	5.280	2.22					
II <sub>7</sub>	25.5	*	5.421	3.48	XV <sub>1</sub>	36.0	11.0	5.002	1.67
II <sub>8</sub>	22.5	5.0	4.745	3.06	XV <sub>2</sub>	24.0	5.5	4.930	3.13
III <sub>1</sub>	31.5	*	5.722	.41	XVI†	73.0	17.0	7.496	1.26
III <sub>2</sub>	17.7	6.5	5.977	1.47	XVI†	63.0	13.5	6.424	2.99
III <sub>3</sub>	17.0	3.5	2.788	1.71	XVI <sub>1</sub>	31.0	9.3	5.732	2.44
III <sub>4</sub>	17.5	3.0	3.134	1.96	XVI <sub>2</sub>	26.5	6.0	5.160	4.09
III <sub>5</sub>	19.7	2.5	2.802	2.50					
III <sub>6</sub>	16.0	3.4	3.188	3.91	XVII <sub>1</sub>	34.2	10.0	6.193	1.52
III <sub>7</sub>	20.0	4.3	3.607	2.44	XVII <sub>2</sub>	27.0	6.0	2.809	4.36
III <sub>8</sub>	21.2	4.0	3.893	4.68					
IV <sub>1</sub>	18.0	3.4	3.563	2.02	XVIII <sub>1</sub>	21.0	6.0	4.383	1.41
IV <sub>2</sub>	Lost	—	—	—	XVIII <sub>2</sub>	26.0	6.3	3.815	1.23
IV <sub>3</sub>	26.5	5.5	5.312	1.14	XIX	23.0	4.4	3.604	1.77
IV <sub>4</sub>	24.5	5.4	5.182	2.19	XIX <sub>1</sub>	29.5	6.9	3.938	2.60

\* All used.

† Check.

‡ Cut surface.

repeated, the amount of sugar present is increased, although the respiration may fall away. The two processes, while coincident, are not necessarily proportional. The glucose is available for respiration if material is needed, but the plant accomodates itself to repeated injury or becomes fatigued, and less and less goes into the waste form of wound carbon dioxide.

#### SUMMARY

1. Cutting a potato tuber causes a rapid rise in the rate of respiration during the second and third days after the injury, with a subsequent gradual fall in the curve until the normal rate of the uninjured tubers is attained. If the cutting is repeated in 5-7 days the curve does not rise as high. Repeated cuttings lower the curves until finally the tuber will not respond.

2. Shortening the interval between cuttings to 3-4 days will not step up the effect, the injury stimulus disappears even more quickly.

3. Lengthening the interval between injuries to 11-12 days gave the plant an opportunity to recover, and the curves obtained by the respiration following the later cuttings more nearly approached that attained after the first cutting.

4. If the cut surface remains uninjured for a considerable length of time (18-22 days) the tuber regains its ability to respond to this type of stimuli.

5. A crack in a tuber causes a response almost as large as a cut of twice the area.

6. Bruising the tuber will not induce any marked increase in the rate of respiration.

7. Tubers infected with the scab or rot organisms give a much higher rate of respiration than clean ones.

8. The capacity of response to injury is not lost in very old tubers from storage (15 months).

9. The respiration curve, when examined in detail, shows a maximum the second day while the temporary stoppage layer is in deposition in the outer layer of cells. This first maximum may be followed by a drop in the curve, followed by a second maximum the third or fourth day while the cells of the new cork cambium are dividing. The curve drops away as the new cork layer is completed. Occasionally, after injury, the respiration may drop for twelve hours before rising to the first maximum.

10. The loss in the respiration curves resulting from the later cutting could be easily compensated by increasing the cut surface to 150, 180, 210 per cent of the original area by not only renewing the original injury but adding to it by cutting one or more of the pieces in two. The respiration curve rose even higher than the original one.

11. Deep cutting (removal of a layer 1 cm. thick) did not produce a respiration curve equal to the original one. The injury fatigue apparently extends through a considerable portion of the tuber. Cutting the other end of the tubers produced a curve as high as at first, indicating that the fatigue did not include all the tuber.

12. A cavity on the inside of the tuber produced a marked rise in the respiration, and seemed to have practically the same effect as an external wound.

13. A large part of the effect of the injury on respiration can be removed by washing the cut surface in running water for 1-3 hours. The respiration activators, or the substances on which they act, seem to be water soluble, and to be located mostly in the external cells.

14. The injured tubers respire intramolecularly in an atmosphere of hydrogen, but this respiration is small in amount and does not respond to the injury stimulus.

15. Chemical analyses of the slices cut from the tubers at various times showed an increase in the reducing sugar content. The gradual lowering of the rate of respiration after wounding is coincident with the rise in sugars. The two processes, therefore, do not seem to be dependent on each other.

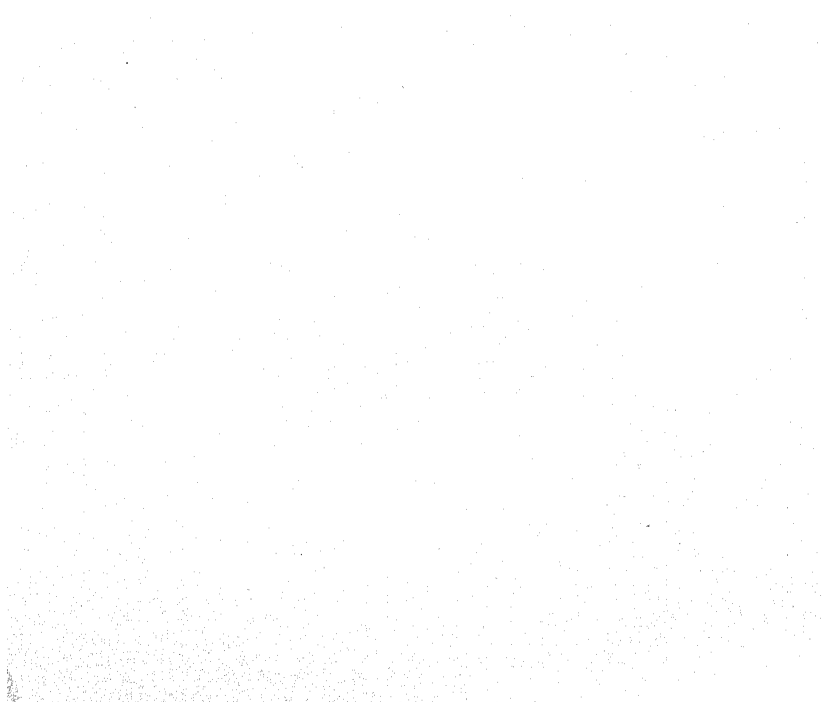
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## Studies of West Indian plants—XIII

NATHANIEL LORD BRITTON

### 74. UNDESCRIBED SPECIES FROM CUBA

#### *Andropogon virginicus graciliformis* Léon, var. nov.

Lower sheaths not equitant, rather terete, glabrous; culms slender and cylindrical from the base, usually producing one or two flowering branches, only from the two or three upper nodes; nodes of the inflorescence long pilose; blades at first narrowly involute or conduplicate from base to apex, becoming flat and flexuous in age, only about 1.5 mm. wide, devoid of long hairs towards the base; spathes becoming reddish, the upper ones 6 to 9 cm. long, much longer than the racemes.

Gravelly soil, Sabana de Motembo, Santa Clara, Cuba (*Léon and Loustalot 11343*). This variety may be readily recognized by its basal sheaths not strongly compressed and equitant as those of typical *Andropogon virginicus* and in its long and very slender stems and narrow leaves. The type specimen is preserved in the herbarium of Colegio de la Salle, Vedado, Havana. We are indebted to Prof. A. S. Hitchcock and Mrs. Agnes Chase for suggestions and help.

#### *Paspalum motembense* Léon, sp. nov.

Perennial, glabrous, with a rhizome; culm erect or nearly so, glabrous, about 60 cm. high, simple, the nodes glabrous; sheaths much longer than the internodes, glabrous, striate, compressed, the basal ones overlapping; ligule a delicate membrane 1-1.5 mm. long; blades 20 to 30 cm. long, up to 5 mm. wide, glabrous on both surfaces, scaberulous on the border, conduplicate, acuminate at the convolute apex; panicle short exerted, 15 cm. long, the rachis angled; racemes several, ascending, 2 to 5 cm. long, erect or falcate, their rachis about 1 mm. wide, glabrous, scaberulous on the border; spikelets in pairs, elliptic, 2.5 mm. long, 1.2 to 1.4 mm. wide, the pedicels scaberulous, one of them 1 mm. long, the other two-thirds as long; glumes covering the fruit, thin, glabrous, brownish-yellow; second glume 5-nerved, short pointed; sterile lemma faintly 3-nerved, fruit brown, slightly rugose, shining.

Grassy place, Sabana de Motembo, Santa Clara, Cuba (*Léon and Loustalot 9354*). Related to the South American *P. hydrophyllum* Henr., a much more robust plant, and to the Cuban *P. Wrightii*; this is taller and stiffer than *P. motembense*, from

which it differs also in its aquatic habit and wrinkled spikelets. The type specimen is preserved in the Herbarium of the New York Botanical Garden.

***Aristida Pradana* Léon, sp. nov.**

Perennial, culms cespitose, 60 to 80 cm. long, erect, wiry, somewhat flattened, simple and naked, the upper leaves of the culms, if any, reduced to the elongate, strongly adherent and mostly bladeless sheath; ligule a short ciliate membrane less than 0.5 mm. long; blades firm, closely involute from the base, with usually a few hairs on each side of it, scaberulous on the upper surface, glabrous beneath, flexuous, often splitting, up to 60 cm. long or more; panicle 25 to 35 cm. long, the branches few flowered, usually with one branchlet at base, at first stiffly erect, finally divergent or spreading, the lower ones distant, up to 8 cm. long, the axis and branches scabrous; glumes unequal, 10 to 12 mm. long, the first caducous, scabrous on the keel, 1-nerved, awn-tipped, the second glabrous, awn-tipped from a bifid apex; lemma 15 to 17 mm. long, including the short pilose callus about 1 mm. long, and the dark colored scaberulous beak; awns unequal, ascending, the central one slightly recurved, 2 to 3 cm. long, the lateral ones somewhat approximate, one-half as long as the central one or little more.

Arid rocky silicious hillside, Peladeros de Jauco, southwest of Baracoa, Oriente, Cuba (*Léon 12299*).

This species is allied to *A. divaricata* Humb. & Bonpl. of Mexico, but may be distinguished by its much longer and closely involute leaves. It is named in honor of Sr. Enrique Prada of Jauco, who helped very efficiently in collecting work. The type specimen is preserved in Colegio de la Salle Herbarium, Vedado, Havana.

***Chloris Morales-Coelloi* Léon, sp. nov.**

Perennial, glaucous; culms cespitose, erect, glabrous, compressed, freely branching from the lower nodes, 50 to 80 cm. tall; sheaths keeled, often longer than the internodes, glabrous, the basal ones overlapping; ligule a short ciliate membrane about 0.5 mm. long, with a few hairs behind on each side; blades flat or conduplicate, glabrous on both surfaces, abruptly pointed at apex, 8 to 20 cm. long, 2 to 4 mm. wide; inflorescence finally long exserted; spikes 5 to 11, mostly 6 or 7, ascending, finally divergent or spreading, straight or recurved, sometimes flexuous, 6 to 9 cm. long; axis of inflorescence 1 cm. long or less, the rachis of the spikes scabrous and densely pubescent at base; spikelets, exclusive of the awns, mostly 3 to 3.4 mm. long, appressed, im-

bricate; glumes unequal, acuminate or awn-tipped, scaberulous on the nerve, the second 3 mm. long, the first more than one-half as long; fertile lemma pale, five times as long as wide, wider in the middle, 3-nerved, villous on the callus, short villous on the marginal nerves from base to apex, and often on the keel, from above the base to the middle, the awn 8 to 12 mm. long; sterile lemma 1 to 2.2 mm. long, wider below the apex than at base, acute at apex, its awn 6 to 10 mm. long; some of the lower spikelets include sometimes a second awned sterile floret.

In sandy ground, at Cajobabo, not far from the mouth of Jojó River, Oriente, Cuba (*Léon 12320*). Named in honor of Colonel Morales Coello of the Cuban Navy, in recognition of his effective help. This species is of *Chloris Sagraeana* relationship, but a much taller plant; it also differs in its longer and broader leaves and more leafy stems. The type specimen is preserved in Colegio de la Salle Herbarium, Vedado, Havana.

***Peperomia Roigana* Trelease, sp. nov.**

A puberulent suberect fleshy herb; stem slender (2 mm.); leaves in crowded whorls above, more separated below, about 4 at a node, somewhat angularly suborbicular or slightly ovate or obovate, obtuse, sessile, rather small (10 × 12–14 × 15 mm.), drying opaque, obscurely 3-nerved by transmitted light and finely pellucid-punctulate; inflorescence unknown.

Mogote de la Bandera, Sierra de Viñales, Pinar del Rio, Cuba, (*Roig & Azcuy 2902*).

***Torrubia Leonis* Standley, sp. nov.**

Shrub 2 m. high, the branches grayish, the branchlets densely brownish-tomentose or in age merely puberulent, evenly leafy, with short internodes; petioles slender, 6–15 mm. long, brownish-tomentose; leaf blades oblong-elliptic or narrowly elliptic, 4–7 cm. long, 1.7–3.3 cm. wide, rounded or very obtuse at apex, obtuse or rounded at base or sometimes acute, subcoriaceous, above deep green, lustrous, with obsolete venation, densely short-villous when young but in age only sparsely short-villous or glabrate, beneath slightly paler, copiously villous even in age with short slender spreading hairs, the lateral nerves very slender and irregular; pistillate peduncles slender, 1–2 cm. long, thinly brownish-tomentose, the inflorescence cymose, few or many-flowered, the flowers sessile; pistillate perianth tubular, 1.5–2 mm. long, with spreading limb, glabrous or nearly so; style exserted.

Type in the herbarium of the New York Botanical Garden, collected on limestone rocks at the top of the Sierra de Anafe, Province of Havana, Cuba, May 6, 1924. (*Brother Léon 11622*.)

There are only a few species of *Torrubia* that have pubescent foliage. The present plant is related to *T. cuspidata*, of Grenada and Trinidad, but differs conspicuously in leaf characters.

***Tounatea cubensis* Britton & Wilson, sp. nov.**

A small tree, the young twigs, petioles, and rachis more or less densely pubescent with dark brown hairs. Leaves odd-pinnate, 1.5–2.6 dm. long, the leaflets 13 or 15, elliptic-lanceolate to elliptic-oblongate, 5–9.5 cm. long, 2–3 cm. broad, short-petioled, the base obtuse, the apex acuminate, finely reticulate-veined on both surfaces; pod ellipsoid, about 4.6 cm. long.

Valley of the San Juan, Pinar del Rio, Cuba (*J. T. Roig 3162*, April 5, 1924, foliage, type; fruit sent by Dr. Roig from Pinar del Rio, June 1925). This is the first record of the genus *Tounatea* in Cuba. In leaf form it approaches *Tounatea caribaea* (Griseb.) Taub. of the Lesser Antilles.

***Bunchosia linearifolia* P. Wilson, sp. nov.**

Shrub about 3 m. tall, with grayish branches. Leaves linear or somewhat linear-obovate, 3–6 cm. long, 2.5–5 mm. wide, rounded at the apex, acute at the base, lustrous above, dull and faintly nerved beneath, the petioles 2–3 mm. long; drupes subglobose, 10–12 mm. in diameter, apiculate.

Type collected in thickets between Jauco and Cajobabo, Oriente, Cuba (*Brother Léon 12062*). Related to *Bunchosia Leonis* Britton & Wilson, of Cuba, but differing in its linear-obovate leaves.

***Byrsonima motembensis* Britton & Small, sp. nov.**

A shrub 2 m. tall or less, with warty-rugose gray bark and red-strigillose twigs. Leaves mainly on the twigs, early deciduous from the branches, the blades cuneate to obovate-cuneate, 2–6 cm. long, obtuse or abruptly short-pointed, grayish-green, somewhat lustrous and with minute scattered hairs above, paler, dull, somewhat reticulate, and with minute scattered hairs beneath, at least during anthesis, short-petioled, the petioles hairy like the twigs when young; panicles raceme-like, peduncled, rather few-flowered, the peduncle and pedicels pubescent like the twigs, but the hairs often early deciduous from the peduncle; bracts shorter than the pedicels, lanceolate; sepals ovate to orbicular-ovate, 3–3.5 mm. long, obtuse, red-pubescent, corolla 12–15 mm. wide; petals 7–9 mm. long, with orbicular-reniform claws, the blade of one smaller and the claw stouter than in the others; filaments clavate, 2–2.5 mm. long; anthers fullv

2 mm. long, blunt or notched at the apex, acute at the base; styles subulate, curved at the apex; fruits not seen.

In thickets of Sabana de Motembo, Santa Clara, Cuba, August 28, 1922 (*Brother Léon and A. Loustalot 11370*).

***Leucocroton pallidus* Britton & Wilson, sp. nov.**

A scurfy-pubescent shrub with slender light brown twigs. Leaves coriaceous, oblong-linear, 5-12 cm. long, 5-10 mm. wide, pale green above, grayish green beneath, mucronate at the apex, obtuse or acute at the base, short-petioled, the midvein impressed above, prominent beneath, the lateral veins rather prominent on both sides, diverging at right angles to the midvein; flowers and fruit unknown.

Forest on Mesa de Prada, Oriente, Cuba (*Brother Léon 11960*). Similar to *Leucocroton saxicola* Britton, but with longer and narrower leaves.

***Pachyanthus Lunana* Britton & Wilson, sp. nov.**

A shrub with ferruginous twigs. Leaves ovate, 5-5.7 cm. long, 2-3.2 cm. broad, glabrous above, pubescent beneath with mostly scattered stellate ferruginous hairs, acute at the apex, subcordate at the base, coriaceous, petioled, 5-nerved, the veins and lateral nerves prominent beneath, slightly impressed above; inflorescence short peduncled; calyx about 4 mm. long, 4 mm. broad, pubescent with shaggy hairs, its lobes oblong, 2 mm. long.

Lomas de Banao, Santa Clara, Cuba (*Antonio Luna 8*). Related to *Pachyanthus Clementis* P. Wilson, of Cuba, from which it differs in its short-ovate leaves.

***Icacorea baracoensis* Britton & Wilson, sp. nov.**

A small tree 5-6 m. high, the twigs and branches glabrous. Leaves oblanceolate, 5-11 cm. long, 1.8-4 cm. broad, obtuse at the apex, cuneate at the base, indistinctly veined, glabrous, the margin entire, short-petioled; branches of the inflorescence corymbiform, the lower pedicels elongated; sepals 5, triangular, acuminate, 0.9 mm. long, 0.8 mm. broad; petals 5, elliptic-ovate or elliptic, 3 mm. long, 2 mm. broad, obtuse at the apex.

On rocks, Sierra de Imias, Oriente, Cuba (*Brother Léon 12256*). This plant differs from nearly all of the Cuban species of *Icacorea* in its corymbiform inflorescence.

***Bumelia Roigii* Britton and Small, sp. nov.**

Tree with gray branches and sparingly pubescent twigs, the shoots sometimes with axillary subulate thorns: leaves persistent;

blades coriaceous, obovate, rhombic-obovate or somewhat cuneate, 3-7 cm. long, rounded at the apex, smooth, glabrous, and shining above, closely tawny-pubescent beneath when young with the hairs fading or deciduous in age, acuminate at the base, short-petioled: flowers not seen: fruit large, apparently about 1.5 in diameter.

Between Cape San Antonio and Morro de Piedras, Pinar del Rio, Cuba, April 13, 1924, (*Roig* 3256, type, and 3257.)

The specimens indicate, by the leaves, a relationship between this species and *Bumelia loranthifolia*; the fruit of *B. Roigii*, however, is much larger than any heretofore found in the former species.

***Maba Leonis* Britton & Wilson, sp. nov.**

A shrub with grayish brown strigose-pubescent twigs. Leaves elliptic to oval, occasionally somewhat ovate, 1.5-3.5 cm. long, 0.8-2.2 cm. broad, spinulose-apiculate at the apex, rounded or obtuse at the base, finely reticulate veined on both surfaces, pubescent beneath with appressed hairs or glabrous; petioles 2-2.5 mm. long; young fruiting calyx lobes suborbicular, 6-7 mm. broad, densely strigose pubescent on the back; ovary strigose-pubescent.

Thickets near Cojimar, Havana, Cuba (*Brother Léon* 6269, type; 5609). Related to *Maba Grisebachii* Hiern, of Cuba, from which it differs in its elliptic or oval leaves which are finely reticulate-veined.

***Neobracea angustifolia* Britton, sp. nov.**

A shrub or small tree, with slender branches, the leafy twigs densely short-pilose. Leaves linear-oblong, subchartaceous, 4-7 cm. long, 8-10 mm. wide, densely tomentose, the venation rather prominent beneath, the apex obtuse, the base narrowed, the petioles 2-3 mm. long; inflorescence few-flowered, peduncled, shorter than the leaves, pilose; calyx-segments lanceolate, acuminate, pilose, about 3 mm. long; corolla purplish, about 1 cm. long.

Rocky soil between Santa Cruz and Los Coyuelos, Pinar del Rio, Cuba (*Roig* 3227). In general appearance this plant is similar to *Neobracea bahamensis* Britton, of Cuba and the Bahamas, but differs in its much smaller corolla and narrower leaves.

***Tournefortia Roigii* Britton, sp. nov.**

Shrubby, the twigs and inflorescence sparingly pubescent with short white appressed hairs. Leaves oblong to oblong-



elliptic, 4-7 cm. long, dark green above, pale green beneath, sparingly pubescent with short white hairs on both sides, the venation prominent beneath, the apex acuminate, the base cuneate, the petioles short; branches of the slender-peduncled cymes many-flowered, 6-9 cm. long; sepals linear-lanceolate, acuminate, 3 mm. long; corolla-tube nearly glabrous, 4 mm. long, its lobes ovate, about 1 mm. long; anthers ovoid, 1 mm. long; berries globose, about 6 mm. in diameter.

Open places, El Gato, Pinar del Rio, Cuba (*Roig 3208*). Related to *Tournefortia bicolor* Sw., of the West Indies and continental tropical America, but differing in the prominent venation of its lower leaf surface.

***Tournefortia Leonis* Britton & Wilson, sp. nov.**

A vine 3 m. or more long, the young twigs densely pubescent with appressed brownish hairs. Leaves narrowly oblong or oblong-elliptic, 2-4.3 cm. long, 4-10 mm. wide, acute at the apex, nearly glabrous above or with short scattered appressed hairs on both sides, the petioles 2-3 mm. long; cymes short-peduncled; calyx about 1 mm. long, appressed-pubescent, its narrowly lanceolate lobes acute; corolla-tube 2 mm. long, the linear lobes 2.5 mm. long; fruit depressed, about 4 mm. broad, glabrous.

Type from coastal thickets, Jauco Abajo, Oriente, Cuba (*Brother Léon 12383*); also collected between Sabana and Maisi (*Shafer 7910*). Related to *Tournefortia volubilis* L., from which it may be distinguished by its much narrower leaves.

***Callicarpa Roigii* Britton, sp. nov.**

A shrub, the stout branches, petioles, inflorescence and lower leaf-surfaces densely grayish-floccose, becoming glabrate when old. Leaves broadly ovate, submembranous, 6-10 cm. long, strongly pinnately veined, serrulate, the apex acute or acuminate, the base obtuse or rounded, the stout petioles 8-15 mm. long; cymes stout-peduncled, densely many-flowered, shorter than the leaves, 4-7 cm. broad; pedicels very short; calyx campanulate, subtruncate, 1 mm. long; corolla white, about 3 mm. broad, its lobes rounded; fruit apparently black, about 4 mm. in diameter.

Rocky places, Bolondron, Pinar del Rio, Cuba (*Roig 3220* type, in flower April, 1924); Punta de la Jaulu (*C. Wright 3169*, in fruit, Dec. 22). Referred by Grisebach to *C. acuminata* Kunth. Related to *Callicarpa acuminata* H.B.K. of Mexico and Central America.

***Solanum lomensis* Britton & Wilson, sp. nov.**

A tree 6-8 m. high, the twigs and branches armed with brownish prickles 2-4 mm. long, densely hispid with ferruginous, long-stalked, stellate hairs. Leaves broadly elliptic, 7-14 cm. long, 4.5-8.5 cm. broad, acute to short-acuminate at the apex, rounded at the base, coarsely sinuate undulate, rather dull and with few slender prickles above, the midvein and primary veins impressed, densely hispid beneath with stalked stellate hairs, the midvein and primary veins often rather prominent; petioles 7-15 mm. long; inflorescence 5-10 cm. long; calyx about 2 cm. in diameter; corolla 3.8 cm. in diameter; anthers 6-6.5 mm. long, attenuate at the apex.

Loma San Juan near Loma del Gato, Oriente, Cuba (*Brother Léon* 2520, type; 12353). Similar to *Solanum Gundlachii* Urban, of Cuba, but differing in its indumentum.

***Rondeletia myrtacea* Standley, sp. nov.**

Shrub 3-4 m. high, the branches terete, reddish brown or grayish, with short or elongate internodes, glabrous or when young sparsely puberulent; stipules broadly triangular, 1.5-2 mm. long, subulate-acuminate, sparsely short-pilose near the apex; leaves opposite, the petioles 2-10 mm. long, glabrous; leaf-blades very variable, ovate-elliptic to oval or oblong-elliptic, 2-6.5 cm. long, 1-3 cm. wide, rounded or obtuse at apex, rounded to cuneate at base, coriaceous, glabrous, lustrous above, the venation prominulous, beneath dull, the costa slender, salient, the lateral nerves about 6 on each side, straight, arcuately anastomosing remote from the more or less revolute margin; inflorescences terminal, few or many-flowered, 3-6 cm. long, cymose-paniculate, the pedicels slender, mostly 3-10 mm. long, glabrous or with a few minute appressed hairs; lower bracts leaf-like, 3-7 mm. long, elliptic or broadly ovate, the upper linear or lance-subulate; bractlets subulate, 1-1.5 mm. long; hypanthium glabrous or with a few minute appressed hairs; calyx-lobes 5, 1.5-2 mm. long, oblong-spatulate, contracted below, rounded or obtuse at apex; corolla-tube 5-6 mm. long, ampliate above, pilose with minute whitish erect-patent hairs, the 5 lobes orbicular, 2-2.5 mm. long; capsule subglobose, 3-5 mm. long, glabrous or glabrate; seeds 1-1.5 mm. long, compressed, yellow-brown, coarsely reticulate, attenuate at each end.

Type in the herbarium of the New York Botanical Garden, collected in gravelly soil near Jauco, Mesa de Prada, southern Baracoa region, Cuba, July 17 to August 4, 1924 (*Brother Léon* 11966). The following collections, from the same region, also belong here: Mesa de Prada, *Léon* 11946; Jauco Arriba, *Léon* 12016.

In general appearance this is very similar to *R. Ekmanii* Britton & Standley, but that species differs in having narrow calyx-lobes which are broadest at base.

***Rondeletia ingrata* Standley, sp. nov.**

Shrub 4 m. high or less, the branches stout, terete, blackish or gray, the branchlets densely pilose with short ascending hairs, the internodes 2-4 mm. long; stipules triangular or broadly triangular, 1.5-2 mm. long, acute, erect, sericeous; leaves opposite, the petioles stout, 1.5-3 mm. long, minutely grayish-pilose; leaf-blades oblong-elliptic, 6-15 mm. long, 3-7 mm. wide, obtuse or rounded-obtuse at apex, obtuse at base, thick-coriaceous, with revolute or subrevolute, much thickened margins, green above, the venation obsolete, when young densely pilose with minute appressed hairs, in age glabrate, beneath covered with a very dense, minute, grayish tomentum, along the nerves sericeous with longer hairs, the costa and lateral nerves elevated, the veins prominently reticulate; inflorescences axillary, usually 3-flowered, sometimes 1-flowered, the peduncles stout, 2-3 mm. long, the flowers sessile; bracts and bractlets deltoid; hypanthium densely tomentose; calyx-lobes usually 5, sometimes 4, oblong-ovate, obtuse, 1.5 mm. long; corolla not seen; capsule globose, 3 mm. in diameter, densely tomentose; seeds minute, compressed, brown, exalate.

Type in the herbarium of the New York Botanical Garden, collected on dry gravelly hills, at Cajobabo, valley of the Río Jojó, southern Baracoa region, Cuba, July 17 to August 4, 1924, (*Brother Léon 12415*). Also collected in the same region, at Jauco Arriba (*Brother Léon 11865*).

Related to *R. camarioca* Wright and, according to description, to *R. Norlindii* Urban. The former differs in the velvety pubescence of the upper leaf surface; the latter in its 1-flowered peduncles and smaller leaves, glabrous on the upper surface.

***Rondeletia gaultherioides* Standley, sp. nov.**

Shrub, the stout branches terete, dark red-brown, with elongate internodes, when young densely pilose with long ochraceous erect-patent hairs; stipules lance-ovate, 5-8 mm. long, acuminate, persistent, densely appressed-pilose; leaves opposite, the petioles stout, 3-5 mm. long, pilose with subappressed hairs; leaf-blades ovate-oval to oblong-elliptic, 3-10 cm. long, 1.7-5.5 cm. wide, abruptly acute to obtuse at apex, rounded or shallowly cordate at base, thick-coriaceous, somewhat lustrous above, when young sparsely appressed-pilose but soon glabrate, the costa impressed, the other venation prominulous, beneath dull, sparsely

pilose with slender, closely appressed hairs, more densely pilose along the nerves, the costa salient, the lateral nerves very prominent, 5 or 6 pairs, nearly straight, ascending at an angle of  $45^{\circ}$ , arcuately anastomosing near the margin; inflorescence terminal, subsessile, 1-flowered, subtended by lanceolate to rhombic-ovate bracts, these longer than the capsule, appressed-pilose; capsule subglobose, 5 mm. long, densely appressed-pilose, the 4 persistent calyx-lobes (imperfect) oblong, 7 mm. long, densely appressed-pilose outside.

Type in the herbarium of the New York Botanical Garden, collected on rocky banks of Arroyo Bayajá, Sierra Maestra, south of Nagua, Oriente, Cuba, August 8, 1922 (*E. L. Ekman 14759*).

The foliage of this plant is suggestive of *R. correifolia* Griseb., but the form of the inflorescence is unlike that of any of the Cuban species of *Rondeletia*.

#### 75. UNDESCRIBED SPECIES FROM TRINIDAD

##### *Gravisia aripensis* N. E. Brown, sp. nov.

Leaves 60–75 cm. or more long,  $4\frac{1}{2}$ –5 cm. broad, broadly strap-shaped, concave; sides parallel up to about 5–6 cm. below the apex, where they incurve to an acute and shortly mucronate point; margins armed with small horizontally spreading pale brown (? green when alive) prickles 1 mm. long and 3–5 mm. apart; surface glabrous, very minutely punctate (not lepidote). Scape 13–14 cm. long in the only specimen seen, 7 mm. thick, clothed with light brown wool and bearing two glabrous sheathing acute bracts 5–7 cm. long, and terminating in a dense subglobose inflorescence 7–8 cm. long and 7–8 cm. in diameter, composed of 4–5 spherical bright pink flower-heads, each about 4 cm. in diameter and sessile in the axil of a large elliptic obtuse mucronate pink outer bract 5–6 cm. long and 4–5 cm. broad. Flowers numerous in each cluster or head. Bracts about 3–3½ cm. long, 10–15 mm. broad, ovate-lanceolate, acute, tipped with a spine 3–4 mm. long, slightly woolly or becoming glabrous. Sepals 15 mm. long, spine-tipped, the lower two-thirds having unequal membranous inrolled margins, one margin about 3 mm. broad and the other 1 mm. broad and more acutely narrowed into the spine than the broad margin, woolly. Petals not seen in perfect condition, but apparently about as long as the sepals and lanceolate. Stamens about three-fourths as long as the petals, absent in two flowers examined; anthers  $3\frac{1}{2}$  mm. long, versatile, oblong, with a blunt mucro. Style rather shorter than the stamens; stigma entire.

Trinidad: Heights of Aripo, growing on trees, Jan. 1922 (*Broadway 9917*).

I have placed this very distinct plant under *Gravisia* because in general appearance it seems more nearly allied to *G. aquilega* Mez, than to any other in the Kew Herbarium. But the flowers are not in a sufficiently good state to admit of complete details being given. I could not, however, detect any ligules upon the petals, nor did I find any pollen to examine. The habit of its inflorescence would seem to distinguish it from *Aechmea*. [N. E. BROWN.]

***Piper maraccasense* Trelease, sp. nov.**

Flowering internodes brown hirsute-tomentose; leaves sub-elliptic-oblong, somewhat acuminate, equilaterally rounded or barely subcordulate at base, rather small ( $3.5-4.5 \times 9-11$  cm.), pinnately nerved from below about the upper third, the rather prominent nerves  $5-7 \times 2$ , glabrous and somewhat glossy above and finally bullate, brown appressed-hairy on the nerves beneath; petiole short (5-scarcely 10 mm.), scarcely winged, densely brown-hairy; spikes opposite the leaves, rather slender and short (scarcely  $4 \times 40$  mm.); peduncle short (5 mm.), brown-subtomentose; bracts concave-inflexed, hairy; flowers sessile, perfect; stigmas 3, sessile, berries subglobose, indented, glabrous.

Maraccas, Trinidad, (Trinidad Bot. Gard. Herb. 2613, collected in 1845). Type at the New York Botanical Garden).

***Coccolobis quadrifida* Britton, sp. nov.**

A small tree with stout somewhat flexuous, glabrous twigs. Leaves elliptic, coriaceous, strongly reticulate-veined on both sides, glabrous, 7-10 cm. long, shining above, the apex acute, the base obtuse, the stout petiole 1-1.5 cm. long; ocreae membranous, about 12 mm. long or shorter, their lobes acuminate; spikes slender, densely flowered, 6-8 cm. long, numerous, glabrous, occasionally forked at the base, short-peduncled, 6-10 cm. long; flowers sessile; buds about 2 mm. long; sepals 4; stamens 8, white; stigma 2-cleft.

Tocuche, Trinidad (*Trinidad Herbarium* 11012, collected by R. O. Williams April 28, 1925).

***Elsota lophosoma* Blake, sp. nov.**

Branches and branchlets densely and softly pilosulous with antorse-curved, yellowish or in age fuscous hairs; leaves oval-ovate or oval-oblong, obtuse, papery, softly incurved-pilosulous on both sides, more densely so beneath; racemes terminal; fruit about 6 cm. long, sordid-pilosulous, the cell subglobose, about 1 cm. long, each side covered with about 5

elevated anastomosing thick blunt crests (about 2 mm. high), the upper margin of the cell bearing a repand-denticulate wing-margin about 3 mm. high, this truncate or emarginate at base of proper wing and not decurrent on it, the proper wing obliquely obovate, about 5 cm. long, 7 mm. wide at base, 2 cm. wide above the middle.

Shrub, doubtless scandent, branching; petiolar glands slender, peiziziform, 0.5 mm. long; leaves distichous; petioles densely yellowish-pilosulous, 1-2 mm. long; leaf blades 2.5-4.5 cm. long, 1.7-2.3 cm. wide, rounded at base, dull brownish-green above, slightly paler beneath, loosely prominulous-reticulate on both sides, the lateral veins 7-10 pairs; racemes terminating leafy branches, solitary, in fruit 4 cm. long or less, pubescent like the stem, the bracts deciduous; flowers unknown; proper wing of fruit curved at apex, repand on lower margin, with numerous subparallel veins.

Trinidad: Road to Maraccas Bay, 10 July 1924, (*R. O. Williams, W. G. Freeman, and E. E. Cheeseman 11246*; type in herbarium of Royal Botanic Gardens, Trinidad and Tobago; photog. and fragm. in U. S. Nat. Herb., and in Herb. N. Y. Bot. Gard.).

A species allied to *Elsota coriacea* (Bonpl.) Blake and to *E. sylvestris* (Schlecht.) Kuntze, and characterized primarily by the numerous thick blunt crests of the fruiting cell. The description of *Securidaca tenuifolia* Chod., of Trinidad, suggests this species, but a fruit received from Berlin of Trinidad Bot. Gard. Herb. no. 2703, described by Chodat as a velvety form of *S. tenuifolia*, proves to be entirely different from the fruit of *E. lophosoma*.

*Pedilanthus ierensis* Britton, n. sp.

Stem glabrous, tall, erect, up to 2 m. high. Leaves broadly ovate, rather thin, glabrous, the larger ones 8-15 cm. long, 5-10 cm. wide, the venation widely spreading, the apex bluntly acute, the base narrowed, the stout petiole about 1 cm. long or shorter, the midvein not flanged beneath; flowers similar to those of *P. tithymaloides* but smaller.

Penal Rock Road, Trinidad (*Britton, Hazen and Mendelson 1093*). In flower, March 28, 1920.

*Metastelma Freemani*, N. E. Brown, sp. nov.

Stem about 1 mm. thick, with internodes 10-20 mm. long, terete, puberulous along two opposite rather broad lines with strongly curved very short hairs. Leaves opposite, apparently

slightly deflexed; petiole 1-1½ mm. long, minutely puberulous; blade 9-13 mm. long, 4-8 mm. broad, elliptic or elliptic-oblong, obtusely rounded at both ends or slightly and broadly cuneate at the base, minutely apiculate at the apex, entire, glabrous on both sides. Flowers in small branching or simple axillary racemes 3-10 mm. long, minutely bracteate, and minutely puberulous on the axis, pedicel and calyx. Pedicels ½-1 mm. long. Calyx-lobes 1 mm. long, oblong, rounded at the apex. Corolla 5-lobed; tube 1 mm. long; lobes 1½ mm. long, oblong, subacute, with thickened microscopically puberulous margins within, glabrous on the back. Coronal lobes 5, arising from the sinuses between the corolla-lobes, about 1 mm. long, linear-lanceolate, acute, bent outwards just below the middle and upcurved at the apex. Staminal column 1½ mm. long, arising shortly above the base of the tube of the corolla and exerted from it, much dilated at the truncate apical part. Fruit not seen.

Balandra Bay, Trinidad, (*W. G. Freeman 11310*).

The genus *Metastelma* is one that urgently requires a thorough revision, for as understood by modern authors and as defined in Bentham & Hooker, *Genera Plantarum* 2: 755, it comprises three very distinct types of coronal structure, which in other parts of the order are held to constitute generic distinction, and there seems no valid reason why these differences should not be considered of generic value in this case. The three types are: 1, Plants with the coronal lobes inserted at the sinuses of the corolla. 2, Plants with the coronal lobes inserted at the base of the staminal column. 3, Plants with the coronal lobes inserted at the top of the staminal column.

Originally the genus *Metastelma* was founded by Robert Brown upon a plant collected in the Islands of St. Croix and St. Christopher, having the coronal lobes inserted at the sinuses of the corolla (type 1). This plant (*M. parviflorum* R. Br.) I find upon comparison with the type to be identical with *Thompson 499*, collected on St. Croix, and *M. Freemani*, above described, has the same type of structure and undoubtedly is a true species of *Metastelma*. But modern authors seems to have understood type 2 as being the typical structure of *Metastelma*. While K. Schumann in Engler, *Pflanzenfamilien* 4<sup>2</sup>: 229 has placed plants having the typical structure of *Metastelma* (i. e. type 1) under the genus *Irmischia*, founded by Schlechtendahl in *Linnaea* 19: 738 upon a Mexican plant I have not seen, but which from description may prove to be a typical *Metastelma*; in which case *Irmischia* will rank as a synonym of *Metastelma*.

As to types 2 and 3 mentioned above, I consider that these should be separated from *Melastelma* and made to constitute separate genera. [N. E. BROWN.]

***Jacquemontia elongata* Britton, sp. nov.**

A slender vine, up to 5 m. long or longer, the branches and peduncles appressed-pubescent. Leaves ovate, membranous, slender-petioled, glabrous or nearly so, about 7 cm. long or shorter, the apex acute or acuminate, the base cordate, rounded or subtruncate; peduncles 5-10 cm. long, much longer than the petioles; inflorescence subcapitate, several-flowered; corolla blue to lavender, about 1.5 cm. in diameter.

Thickets, Trinidad, Tobago and northern Venezuela. Type from Manzanilla, Trinidad (*Britton 2191*). This plant has been referred to *J. pentantha* (Jacq.) Don.

***Columnnea tocoensis* Britton, sp. nov.**

Stems rather short, simple or little branched, clustered, 1.5 m. long or less; long, appressed, pubescent. Leaves oblong, 3-4 cm. long, appressed-pubescent, obtusish. Peduncles densely pubescent, 1.5 cm. long or shorter; calyx densely pubescent, about 8 mm. long, its lobes lanceolate, acutish; corolla yellow, about 4 cm. long, loosely villous with nearly white hairs, its slender tube about twice as long as the limb.

Pendent on forest trees, Trinidad. Type from Toco Road, near Valencia (*Britton, Hazen and Mendelson 1785*). This is, perhaps, the plant referred by Grisebach to *C. scandens*.

***Columnnea aripoensis* Britton, sp. nov.**

Branched, the branches rather stout, densely appressed-pubescent, 2 dm. long or longer. Leaves oblong, short-petioled 2.5-3.5 cm. long, finely appressed-pubescent, obtuse; peduncles densely villous, 8-12 mm. long; calyx 10-12 mm. long, appressed-pubescent, its lobes ovate, acute; corolla red, 3-4 cm. long, loosely pubescent with long jointed hairs, its tube gradually expanded above, about twice as long as the limb.

On trees, Heights of Aripo, Trinidad. (*Britton and Freeman 2340*, type).

***Diapedium aripoense* Britton, sp. nov.**

Branching, strigillose, 1-1.5 m. high. Leaves elliptic-ovate, membranous, slender-petioled, rather strongly veined, long-acuminate at the apex, narrowed at the base, the lower about 2 dm. long, the upper much smaller; inflorescence several-flowered,



short-peduncled or nearly sessile; corolla red, villous, slightly curved, about 3 cm. long, its lobes about 6 mm. long.

Heights of Aripo, Trinidad. (Trinidad Herbarium 9860, coll. *Broadway*, Jan. 10-26, 1922.)

***Chimarrhis microcarpa* Standley, sp. nov.**

Branchlets stout, obtusely angulate, ochraceous, glabrous, the internodes 5-13 mm. long; stipules lance-oblong, attenuate, 3 cm. long, thin, brown, glabrous, caducous; leaves opposite, the petioles slender, glabrous, 2.5 cm. long; leaf blades elliptic, 11-19 cm. long, 5-8 cm. wide, acute, at base acute and decurrent upon the petiole, membranaceous, glabrous, the lateral nerves about 10 pairs, arcuate, laxly and irregularly anastomosing close to the margin; inflorescences axillary, cymose-paniculate, half as long as the leaves, 4-5 cm. long and broad, many-flowered, the peduncles slender, 3.5-5.5 cm. long, thinly puberulent, the pedicels 1-2 mm. long; capsules subglobose, 2 mm. long, obtuse at base, broadly rounded at apex, slender-costate, dark red-brown, glabrous, crowned by the low persistent calyx.

Type in the herbarium of the New York Botanical Garden, collected at Maraval, Trinidad, in 1904 (*I. Dannouse 6946*).

Because of the incompleteness of the available material, there is some doubt as to the proper reference of this plant to *Chimarrhis*, but it agrees well with that genus in the characters exhibited by the specimen studied.

76. AN UNDESCRIBED TREE OF PORTO RICO

***Paralabatia portoricensis* Britton & Wilson, sp. nov.**

A tree 15 m. or more high, the twigs clothed with appressed ferruginous hairs. Leaves oblong-lanceolate, 6-11 cm. long, 2.5-4 cm. wide, acute or obtuse at the apex, rounded or somewhat acutish at the base; glabrous above except on the midvein, loosely pubescent beneath with rather long whitish hairs, the petioles about 1.5 cm. long; calyx-lobes 5, oblong-elliptic, 2 mm. long, pubescent on the back; corolla-lobes broadly elliptic to oval, 1.5 mm. long, unappendaged; staminodes filament-like; ovary 2-celled.

On limestone hills, northern Porto Rico. Type from Dominiguito, near Arecibo (*H. T. Cowles 702*).

Related to *P. dictyoneura* (Griseb.) Pierre, of Cuba, but differing in the pubescence of the petioles and lower leaf-surfaces.



## The Marsileas of the western United States

MARGARET STASON

*Marsilea vestita* Hooker and Greville is a widespread and variable species, occurring throughout the western and Pacific United States. It is subterrestrial or aquatic, occurring on the borders of marshy ponds which become dry as the season advances. Until 1902, when *Marsilea oligospora* Goodding was described, all material from this range was referred to *M. vestita*.

*Marsilea vestita* Hooker and Greville (Icones Filicum 2: pl. 159. London, 1831) as described by Baker,<sup>1</sup> is a subterrestrial, tufted or widely creeping plant, with petioles 1-6 inches long, thinly clothed with appressed brown hairs, outer edges rounded and entire. The pedicels are short, erect, solitary, adnate to the upper part of the base of the conceptacle. The conceptacles are horizontal, persistently tomentose, unbordered, one-sixth to one-fifth inch long, with basal teeth prominent, and sori numbering about fifteen. The type locality of the species is the Columbia River region. The range of this species is given by W. R. Maxon in Abrams' Illustrated Flora of the Pacific States<sup>2</sup> as from British Columbia to southern California, east to South Dakota, Kansas, Oklahoma, and Texas.

*Marsilea oligospora* Goodding,<sup>3</sup> with Jackson Hole, Wyoming, as a type locality, is described as a plant 4-7 centimeters high, with leaflets woolly or becoming glabrous, 6-10 millimeters long, 3-7 millimeters wide, sporocarp solitary, 4-6 millimeters long, 4-5 millimeters wide, covered with long, straight and appressed, rarely somewhat woolly pubescence, raphe short, lower tooth short and blunt, upper tooth a mere rounded papilla or wanting, peduncle 5-8 millimeters long, sori 5-8 in each valve of the sporocarp, megaspores oval to rarely oblong, 6-9 in each sorus. The range of this species on the Pacific coast of North America as given by Maxon (loc. cit.) is Washington, Montana, Wyoming, and south in California to Tulare County.

<sup>1</sup> Baker, J. G. Handbook of the fern allies, p. 143. London, 1887.

<sup>2</sup> Abrams, Leroy. An illustrated flora of the Pacific States 1: 34. Stanford Univ. Press, 1923.

<sup>3</sup> Goodding, L. N. Bot. Gaz. 33: 66. 1902.

Here the statement is made that the number of sori to each valve of the sporocarp of *M. vestita* is 9-11, and the number of megasporangia to a sorus 12-20. This is in contrast to *M. oligospora*, described with sori 5-8 in each valve and megasporangia 6-9 per sorus.

On the basis of these distinctions as given by Maxon, an attempt was made to refer a collection of material from San Luis Obispo, California, made by Cecil Stockton, to one of these species. To examine the contents, the sporocarps were placed in boiling water after a small piece of the wall at one end had been cut away. Except when the sporocarp was immature, in the majority of cases the wall split almost immediately, the gelatinous ring bearing the sori emerged and the counting of the sori and megasporangia became a simple matter. From this San Luis Obispo collection, 106 sporocarps were examined. It was found that the upper tooth of the sporocarp ranged from short, though prominent, to long, sharp, and curved, the number of sori from 9 to 20 in each sporocarp, and the number of megasporangia in a sorus from 5 to 24.

A collection of material from Corning, California, made by Mrs. H. M. Hall in July, 1924, was examined in the same manner. In the twenty sporocarps examined, it was found that the upper tooth of the sporocarp varied from sharp and curved to blunt and rounded, in some cases there being a mere vestige of a tooth. Instances of this variation in the upper tooth were found in sporocarps on the same plant. Here the number of sori ranged from 15 to 20 and the megasporangia from 5 to 22, there being no evident correlation between the condition of the tooth and the number of the sori and megasporangia.

Collections of *Marsilea vestita* and *Marsilea oligospora* from several of the western herbaria and from the United States National Herbarium were next examined. For the privilege of studying this material, I am indebted to the kindness of Mr. W. R. Maxon for the loan of the United States National Herbarium collection of western *Marsileas*, to Professor Leroy Abrams of Stanford University for material from the Dudley Herbarium, to Professor P. A. Munz for material from the Pomona College Herbarium, to Professor Aven Nelson for a collection of *Marsilea oligospora* from the Rocky Mountain Herbarium, and to Professor N. L. Gardner for access to the

collection of the herbarium of the University of California. The data collected have been arranged in TABLE 1, in which the following symbols were used: *N* for the United States National Herbarium; *P* for the herbarium of Pomona College; *S* for the Stanford University Herbarium; *R* for the Rocky Mountain Herbarium; and *C* for the Herbarium of the University of California.

It may be seen from this table that in cases where the sporocarps bore a prominent or a sharp tooth, sori were found in numbers from 9 to 23 in each sporocarp, and megasporangia per sorus from 5 to 24. When the tooth of the sporocarp was reduced to a smooth, rounded protuberance the number of sori varied from 11 to 20 and the megasporangia from 1 to 18. It should be noted that in the examination of this material, often some megasporangia were broken from their sori and were lost before they could be counted, and also when the sori did not emerge from the sporocarp it was impossible to count the number of megasporangia. Such cases are left blank in the table.

In addition to the examination of the contents of the sporocarps, hand sections were made of their walls in the region of the tooth. This tooth was found to be a prolongation made up of stone cells, a continuation of the layers of such cells which form the outer wall of the sporocarp. The amount of this tissue varies with the size of the tooth and in cases where no tooth is present there are no extra stone cells developed.

Measurements of megasporangia of both types of sporocarps, those with a sharp tooth and those with none, revealed no differences in size and shape as suggested by Goodding, who observed the megaspores of *Marsilea oligospora* to be oval to barely oblong. There is some slight variation in the size and shape of the megaspores of a single sorus, but measurements of megaspores from several sporocarps of the same collection showed almost exactly the same range in size, length, and width in the two types. There also was no apparent difference in the size of the sporocarps in the two types. They vary in the sharp toothed form from 2 to 7 millimeters wide and from 3.5 to 10 millimeters long, and in the blunt toothed forms from 2.5 to 6 millimeters in width and from 4 to 8 millimeters in length.

The pubescence of the leaflets, petioles, and sporocarps was

found to vary widely in amount in the two types, that of the sporocarps of both being sometimes of appressed hairs and sometimes woolly-tomentose. There was no difference noted in the woolly pubescence occurring at the tufted bases of the plants.

A brief study of the distribution of the western forms of *Marsilea* brought out several interesting facts. *Marsilea oligospora* is attributed by Maxon to the Transition Zone of Merriam's classification,<sup>4</sup> while *Marsilea vestita* is definitely assigned to the Sonoran Zone. My study brought out rather clearly this same general limitation of distribution. It can be seen from the accompanying table, however, that several seemingly notable exceptions were found. In the case of the material from Corning, California, where sporocarps with conspicuous teeth and those with almost none were found, the habitat is definitely in the Sonoran Zone and in the Pacific semi-desert region, as indicated by the map of vegetation areas by Livingston and Shreve.<sup>5</sup> Another instance to be noted is the occurrence of both types of *Marsilea* along the Columbia River in the Great Basin type of vegetation area. This same mixture occurs in Lassen County, California, a district where the Great Basin and the Western Xerophyte forest vegetation areas intermingle. This region is of the Transition Zone. The sharp toothed form is not entirely confined to the Sonoran Zone or to Great Basin, Semi-Desert, or Grassland areas, but is found in the forest areas of western Montana and Colorado. It would seem therefore, that as far as the general vegetation areas are concerned, there is no sharp limitation in the distribution of either type.

Two specimens from central Florida (Eustis County, Florida, *George Nash* 831, and Orange Bend, Florida, *L. M. Underwood* 337, both in the United States National Herbarium and in the University of California Herbarium) are of the *Marsilea oligospora* type but decidedly extra-limital in distribution. Three sporocarps of the first collection and two of the second were examined. In none of these was there evidence of an

<sup>4</sup>Merriam, C. Hart. Life zones of the United States. U. S. Dept. Agr. Bull. 10. Washington, 1898.

<sup>5</sup>Livingston, B. E., and Shreve, F. Distribution of vegetation in the United States as related to climatic conditions. Carnegie Inst. Wash. Publ. 84. 1921.

TABLE I

State	Locality	Vegetation area	No. of sporocarps examined	2nd tooth of sporocarp sharp	2nd tooth of sporocarp blunt	No. of sori per sporocarp	No. of mega-sporangia per sorus	Herbarium
Washington	Columbia River opposite the Wenatchee	Great Basin	1	+		16		N
	Sauvies Island	" "	1	+		18	7-10	N
	Falcon Valley	" "	2		+	17-18	4-11	N
	Sentinel Bluffs	" "	1	+		17		N
	Pullman	" "	1	+		18	9-12	S
	Klichitat Co. along Columbia	" "	2	+		13-14	9-10	S
	Lake Chelan	Grassland	2	+		15-17	9-9	N
Oregon	The Dalles	Great Basin	1	+		18		N
	" "	" "	1	+		17	9-8	N
	" "	" "	1	+		12	8-10	N
	Klamath Falls	" "	1	+		16	6-8	N
California	Columbia River	" "	1	+		19	12-13	N
	Pine Creek	" "	1		+	16	8	C
	Eagle Lake	" "	1		+	11	Undeveloped	C
	Honey Lake	" "	1	+		20	"	C
	Lake Tahoe	Western Xerophyte forest	1		+	16	7-10	N
	Corning	Pacific semi-desert	20	+	+	17-20	6-22	C
	Yolo County	" "	1	+		20	8-12	P
	Dos Palos	" "	1	+		17	7-10	N
	San Luis Obispo	" "	1	+		14	10-12	N
	" " "	" "	106	+		9-20	5-24	C
	Tulare County	" "	1	+		16	14-12	P
	Tulare	" "	2	+		17-18	10-12	P
	Chico	" "	1	+		18	9-13	C
	Galt	" "	1	+		23	12	S
	Emigrant Gap	" "	1	+		20	6	C
	Ramona	" "	1	+		22	8-9	C
	Laguna	" "	2	+		19-16	10-16	P
	Kenworthy	" "	1	+		16	5-9	P
	Cuyamaca Lake	" "	2	+		18-20	10-12	P-C
Montana	West Riverside	" "	1	+		18	9	C
	Blackfoot River	W. X. forest	1		+	15	7-5	N
	N. W. Montana	" " "	1	+		16	10-11	N
Idaho	Weiser	Great Basin	1	+		19		P
Nevada	Winnemucca	" "	1	+		16	8-9	N
Wyoming	Jackson Hole	W. X. forest	1		+	15	1-1	N
	Kendall	" "	2		+	15	9-6	
	Sublette County	" "	1		+	14	6-11	R
Colorado	Sagnache	" "	1	+		15	8-5	N
	Boulder	" "	1	+		20	10-12	P
N. Dakota	Benson County	Grassland	1	+		16		P
S. Dakota	Woolsey	" "	1	+		16		N
Texas	Dallas	" "	2	+		20-19	6-12	N-P
Florida	Eustis Co	S. E. Evergreen forest	3		+	13-18-20	Undeveloped	N-C
	Orange Bend	S. E. Evergreen forest	2		+	15-17	none	C-N

upper tooth. The number of sori varied from 13 to 20. An interesting feature of these sporocarps was the seeming absence of megasporoes. In the Eustis County collection the sporocarps were evidently immature, as shown by the condition of the microsporangia, and in developing sori the microsporangia can be distinguished before the megasporangia are sufficiently developed for recognition. This collection therefore is not conclusive. In the Orange Bend material, however, the microsporangia were well developed and the microspores numerous, but there was no evidence of megasporangia. This is a condition not met with elsewhere in the study of the western material where the sporocarps had reached maturity.

It seems evident that there is no clear cut distinction between the two forms of *Marsilea* that might be spoken of as the *vestita* and the *oligospora* types, in habitat, distribution, form of the plant, pubescence, the number of sori in a sporocarp, the number of megasporangia in a sorus, and no correlation between these factors and the most pronounced variation, that of the tooth of the sporocarp. Even in the character of the tooth, there seems no definite line between forms with the second tooth present and those without, there being all gradations between them. Possibly there may be here in *Marsilea vestita* what Turesson<sup>6</sup> calls an 'Ecospecies,' that is, a widely distributed and variable species in which ecological factors have caused differentiation into two hereditary types. The *oligospora* type may then be termed an 'Ecotype,' and it may be supposed to be a subtype of the Ecospecies which has arisen by response to some possible habitat factors. Such an hypothesis can only be tested by culture methods, and only vaguely suggested by the examination even of more ample herbarium material than was placed at my disposal.

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<sup>6</sup> Turesson, Gote. The scope and import of genecology. *Hereditas* 4: 171-176. 1923.



## Pollen grain morphology in the classification of the Anthemideae

R. P. WODEHOUSE

(WITH TWO TEXT FIGURES)

The spiny character of the pollen grain of the Carduales has long been a matter of comment. It is almost as universal among the Carduales as the more salient characters of the order, such as, for example, the aggregation of the flowers on a receptacle surrounded by an involucre, the union of the anthers into a tube, or the reduction of the calyx to bristles or scales, or its entire absence. This spiny character of the pollen grain of the Carduales can be made to serve admirably in their classification, because the arrangement, shape, and size of the spines varies more or less continuously throughout the order just as do the grosser morphological characters. From an examination of the pollen grains alone it is always possible to tell to which of the three families of the Carduales a species belongs, and nearly always even the tribe. In spite of this, however, pollen grain morphology is rarely used in taxonomic work.

That this spiny character of the pollen grain arose independantly in the Carduales seems probable from its absence in such related families as Lobeliaceae and Calyceraceae. In the Campanulaceae and Dipsacaceae, though minute spines are present, they do not resemble the spines of the Carduales and appear to bear no relation to them. Moreover in the Carduales, this spiny character reaches its best development in the more generalized forms of the Carduaceae, as, for example the Heliantheae, Astereae, and Vernoneae. Needless to say spinyness has arisen independently in many other families, notable among which are the Convolvulaceae and Malvaceae, but in none of these do the spines themselves or their arrangement resemble those of the Carduaceae at all closely. The spines of the Carduaceae may be described as sharp pointed cones which are a part of the exine, not merely articulated or attached to it, and they are partly or entirely covered by the perinium or outer layer of material which overlies the exine. The number, arrangement and size of the spines varies considerably in the

different species of the *Carduaceae*, but they are nearly always geometrically arranged and large enough to constitute the most conspicuous character of the grain. There is only one character of the grain which is more constant for the family, and that is the three germinal apertures situated in the three longitudinal folds which accomodate the changes in volume resulting from the changes in moisture content. This character, however, is not confined to the *Carduales*.

The *Ambrosiaceae* are regarded as an offshoot from the *Carduaceae*. That they may be regarded as more highly developed than the latter is evidenced by the simplification of the floral parts, the extreme reduction or total loss of calyx and corolla, and the extreme reduction of the involucre of the pistillate heads and the accrescence of its bracts to form a pod enclosing the achenes in the genera *Hymenoclea*, *Ambrosia*, *Franseria*, and *Xanthium*. Associated with this simplification of floral parts is a reduction in spinyness of the pollen grains. The amount of this reduction varies somewhat in the different genera. In *Iva* the spines are low and rounded on the top but still prominent. In *Franseria* and *Ambrosia* they are similar but somewhat less prominent, while in *Xanthium* they are reduced to mere specks, and in some species of this genus are scarcely visible.

This reduction of spinyness is entirely in keeping with the anemophilous habit of this family, and probably, therefore, can be regarded as an ecological response to this mode of pollination, as is the case with the reduction of floral parts and the adoption of the dioecious or monoecious habit by most of the species.

On the other hand, the *Cichoriaceae*, which are probably less related to the *Carduaceae* than are the *Ambrosiaceae*, possess the characteristic spines of the *Carduaceae*, though nearly all the other characters of the grains are quite different. Nevertheless the resemblance of the spines of the *Cichoriaceae* to those of the *Carduaceae* is suggestive of a common origin.

In spite of the fundamental quality of the spiny character among the *Carduales*, in three separate tribes of the *Carduaceae* spinelessness has been independently developed. These three tribes are the *Mutisieae*, *Cynareae* and *Anthemideae*. The *Mutisieae* are all spineless, and as far as these studies have gone, no trace of spines has been found. This entire absence of

spines throughout the whole tribe suggests that the group arose from a spineless mutant from the *Carduaceae* stock.

In the tribe *Cynareae* the genus *Centaurea* comprises species exhibiting all degrees of spininess, from its well developed form characteristic of the tribe to its entire absence. This condition suggests that the *Centaureas* are in the process of developing spinelessness.

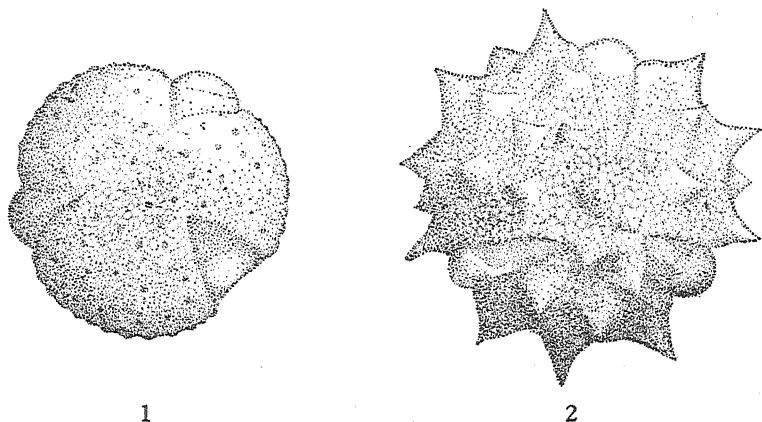


FIG. 1. Pollen grain of *Crossostephium artemisioides* Less. (*Tanacetum chinensis* Gray, *Artemisia chinensis* Vahl., not L.) 20.9  $\mu$  diam. This form of pollen grain is characteristic of the genus *Crossostephium* and the allied genera *Sphaeromeria*, *Vesicarpa*, *Chamartemisia*, *Picrothamnus*, *Artemisia* and *Artemisiastrum*, according to the classification of Rydberg (1916), but is not found elsewhere in the *Anthemideae*.

FIG. 2. Pollen grain of *Tanacetum camphoratum* Less. 29.7  $\mu$  in diameter. This form of pollen grain is characteristic of all species of the genus *Tanacetum*, and is similar to that of all species of the tribe *Anthemideae*, except those of the genera mentioned above.

In the *Anthemideae* the character of spinelessness is of the greatest interest, for it appears abruptly and with little suggestion of transition, and is confined to a group of plants of close but disputed relationships, comprising the following seven genera, according to the classification of Rydberg<sup>1</sup>: *Sphaeromeria*, Nutt., *Vesicarpa*, Rydb., *Chamartemisia*, Rydb., *Crossostephium*, Less., *Picrothamnus*, Nutt., *Artemisia* L., *Artemisiastrum* Rydb. (FIG. 1).

The common character of spinelessness of these seven genera

<sup>1</sup> Rydberg, P. A. *North American Flora* 34: 217. 1916.

would indicate that they had all originated from a common ancestor, a spineless mutant from the Anthemideae stock, or else spinelessness arose independently at two or more points. That the former is more likely seems cogent from the following facts. The pollen grains of these seven genera are almost indistinguishable from each other and show as little variation in size as would be expected within a single genus. Moreover the grosser anatomical characters hint at divergence rather than convergence.

In arranging a classification based on phylogenetic relationships, one is always confronted with the uncertainty of the relative importance of the available characters, so that the classification often becomes largely a matter of personal judgment, varying with different individuals. That this has been the case with these plants in the past is fully manifest by the many classifications that have been used, some of which are still extant, and is permanently recorded in the long lists of synonyms with which most of these species are burdened. Not the least interesting feature about these older classifications of the Anthemideae is the fact that at one time or another, all of the seven genera, except *Sphaeromeria* and *Chamartemisia*, have been regarded as belonging to the genus *Artemisia*, and yet this has been done entirely without reference to pollen grains, which goes to show that there are recognizable anatomical characters which may be interpreted to mean that there is a close relationship between these seven genera. On the other hand, the first three of these genera have more frequently been regarded as belonging to *Tanacetum*.

The difficulty of establishing adequate grounds for separating these genera from each other and from *Tanacetum* becomes apparent from TABLE I, which is taken from Hall and Clements.<sup>2</sup> Here the authors have selected and tabulated in a convenient form, all the significant characters that have been used in the classification or segregation of this group.

In this treatment Hall and Clements state that they prefer to regard the first four genera listed in TABLE I as *Tanacetum*, and the last three as *Artemisia*. The fifth (*Crossostephium*), according to Rydberg, comprises four species, three of which

<sup>2</sup> Hall, H. M., & Clements, F. E. The phylogenetic method in taxonomy. Carnegie Inst. Washington Publ. 326. 1923.

TABLE I

	<i>Anther-tips</i>	<i>Inflorescence</i>	<i>Pappus</i>	<i>Corolla of marginal flowers</i>	<i>Receptacle</i>
TANACETUM	Ovate, obtuse	Cymose or solitary by reduction	Coroniform	Oblique, somewhat ligulate	Naked
VESICARPA	Ovate-lanceolate	Cymose	Wanting	Nearly tubular	Pubescent
SPHAEROMERIA	Ovate, "obtusish"	Cymose or solitary	Wanting	Nearly tubular, slightly if at all oblique	Naked
CHAMARTEMISIA	Subulate	Solitary or two, probably reduced from a cyme	Coroniform	Nearly tubular; no ligules	Naked
CROSSOSTEPHIUM	Subulate	Panicle	Coroniform	Nearly tubular; no ligules; in 2 rows	Essentially naked
PICROTHAMNUS	Subulate	Racemose	Wanting	Short, 2-cleft	Naked
ARTEMISIA	Lanceolate or subulate	Panicked or the panicle reduced and raceme-like or spike-like	Wanting	Tubular, often oblique	Naked or pubescent
ARTEMISIASTRUM	Subulate	Panicked	Wanting	Tubular	Chaffy

are American and one Asiatic. The three American species Hall and Clements prefer to place in *Artemisia*, but the single exotic species they allow to remain, making *Crossostephium* a monotypic genus.

Hall and Clements contend that Rydberg accords generic rank to segregates which are of only specific, or at best, sub-generic value. They state that the eight genera employed by Rydberg really constitute only three, *Tanacetum*, *Crossostephium* (of a single species) and *Artemisia*. Whether Rydberg's genera are too numerous or too few is a matter of little consequence at the present time, for the "question of generic limits is one of criteria" and largely a matter of opinion, and can only be answered when the answer to the question What is a genus? has been agreed upon. Nevertheless the groupings as pointed out by Rydberg are there, and should be recognized in some way. What is a matter of much greater importance is the interrelationship between the groups, for, as Hall and Clements have so frequently emphasized, it is the business of classification to suggest relationships. In this case, however, as we shall see, the evidences of relationship are conflicting, and throw some doubt on the arrangement.

An analysis of the table reproduced above shows that, re-

gardless of pollen characters, there is as much similarity between *Artemisia* and the three genera *Vesicarpa*, *Sphaeromeria*, and *Chamartemisia*, as there is between these three genera and *Tanacetum*. For example, if we compare *Vesicarpa* with *Tanacetum* and with *Artemisia*, using the characters in TABLE I, the anther tips which are ovate-lanceolate, are intermediate. The 'inflorescence' is cymose, thus resembling *Tanacetum*. The pappus is absent, thus resembling *Artemisia*, for in all true species of *Tanacetum* it is said to be present and coroniform. The corolla of the marginal flowers is nearly tubular, thus resembling *Artemisia*, in which they are tubular and sometimes oblique, more closely than it does *Tanacetum*, in which they are oblique and somewhat ligulate. The receptacle is pubescent, thus resembling *Artemisia* more closely than *Tanacetum*, for the latter may be naked or pubescent, while the former is always pubescent. Thus of the five characters most generally used, we have only that of the cymose 'inflorescence,' pointing towards *Tanacetum*. Of the other characters one (viz. the absence of pappus) points definitely towards *Artemisia*. The remaining three are intermediate, but two of them (viz. the tubular corollas of the marginal flowers and the pubescent receptacle) point more strongly towards *Artemisia*. Similarly if we compare *Sphaeromeria* and *Chamartemisia* with *Tanacetum* and *Artemisia*, we find that the evidence of relationship is about equally divided between them, so that, at best, the relationship revealed by the gross anatomical characters is vague and uncertain.

In contrast to this, the spineless character of the pollen grain is perfectly constant. All those of the genera *Vesicarpa*, *Sphaeromeria* and *Chamartemisia* are spineless and none of these species shows any indication of ever having had spines (FIG. 1). In this and their other characters they are nearly indistinguishable from the *Artemisias*. On the other hand they resemble *Tanacetum* only in the possession of the general characters common to the *Anthemideae*. Therefore they do not appear to belong to the genus *Tanacetum* (FIG. 2).

The spinelessness of the pollen grains of these three genera is evidence that they are more closely associated with *Artemisia*, as are also the other spineless grained *Anthemideae* (*Crosso-stephium*, *Picrothamnus* and *Artemisiastrum*), than they are with *Tanacetum*, but it does not necessarily follow that the

whole seven should be considered as a single genus, for there are obviously other characters that serve to separate them from each other; but if one does not choose to recognize these characters as of generic value, then it would be necessary to join all of these genera with *Artemisia*. However, observations in other families show that the pollen characters are more often family characters than generic.

These studies of the pollen grain morphology of the Anthemideae show that the genera *Sphaeromeria*, *Vesicarpa*, *Chamartemisia*, *Crossostephium*, *Picrothamnus*, *Artemisia* and *Artemisiastrum* are all spineless, and consequently probably derived from a common spineless ancestor, because all the other members of the Anthemideae, including *Tanacetum*, are conspicuously spined. Since it is the business of taxonomy not merely to catalogue species, but more especially to show relationships and suggest the probable trend of evolution, the anatomy of the pollen grain is fully as deserving of attention as are any of the more generally used grosser anatomical characters.





## INDEX TO AMERICAN BOTANICAL LITERATURE

1925-1926

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A cytological study of the leaves and growing points of  
healthy and mosaic diseased tobacco plants<sup>1</sup>

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(WITH FOUR TEXT FIGURES AND PLATES 18-29)

Certain conspicuous and characteristically differentiated intracellular bodies have been found in the diseased tissue of plants affected with many of the so-called virus diseases, such as tobacco, wheat, corn, sugar cane, and *Hippeastrum* mosaic, and Fiji disease of sugar cane. These bodies have been variously interpreted as 'degeneration products of diseased nuclei' (Smith 1924), as 'products of disease reaction on the part of the cells' (Palm 1914), as 'fragments of the nuclei of the cells' (Iwanowski 1903), and as 'living amoeboid organisms or perhaps stages in the life cycle of a living organism' (Kunkel 1924, 1925). My studies of the mosaic disease of tobacco have convinced me that these intracellular bodies are not products of the metabolism of diseased cells, as are perhaps the striated bodies which are also regularly associated with the diseased condition in the cells of *Nicotiana Tabacum* and *Solanum aculeatissimum*.

By a cytological study of the mature organs and embryonic regions of both healthy and diseased plants, I have endeavored to determine the distribution in the diseased tissues of the intracellular x-bodies which I have previously described in living mosaic diseased tobacco plants (1924), the method of their distribution at the time of the division of the host cell, and their individual structure and development. I have also endeavored to correlate more exactly than has hitherto been done, the internal cell modifications with the external visible symptoms of the disease, and to determine the relation of the histogenic stage of development of a tissue at the time of its infection to the type of disease symptoms which will develop.

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## I. REVIEW OF THE LITERATURE

The literature of mosaic and related diseases has been reviewed in considerable detail by many authors. Allard (1914b) has reviewed the literature of the tobacco mosaic disease. Wakefield (1921) and Dickson (1922) have reviewed in considerable detail the literature dealing with the symptoms, methods of transmission, and the theories as to the causal agent of the mosaic disease. Excellent reviews of both theoretical and experimental data in regard to the nature of the filterable virus diseases of animals are given by Simon (1923) and MacCollum (1926). McKinney (1925) reviews the current theories as to the nature of the mosaic causal agent. Kunkel (1925) summarizes the established facts as to the nature of the mosaic diseases.

I shall endeavor to bring together, in a more compact summary, the data relating to what seem at present to be the most outstanding phases of the subject. The voluminous literature on mosaic presents a wide range of observational and experimental material. I shall discuss it under the following headings: (1) External symptoms of the tobacco mosaic disease; (2) Symptoms of other mosaic and related virus diseases; (3) Range of hosts susceptible to mosaic and other virus diseases; (4) Strains of mosaic virus; (5) Methods of disease transmission; (6) Carriers of mosaic; (7) Filtration studies; (8) Properties of the virus; (9) Attenuation of the virus; (10) Effects of environment on the expression of mosaic symptoms; (11) Culture of the supposed organism; (12) Nature of the causal agent.

1. *External symptoms of the tobacco mosaic disease*

Mayer (1886) was the first to investigate the tobacco mosaic disease, and to demonstrate its infectious nature. Beijerinck (1899) was the first to fully appreciate the biological significance of the mosaic disease of tobacco. He found that it is propagated as a filterable virus. He believed the disease to be essentially a malady of the chlorophyll bodies. When a plant is inoculated just below the tip, the leaves which develop in the course of the ten day incubation period remain helathy. The young leaves which develop later show, as he describes, a mottled appearance over their entire surface, due to the appearance of numerous yellow spots. The next leaves to develop show dark green blotches along the veins of the second and third order, while

the rest of the blade appears pale green. Sometimes the two colors fade gradually into one another, but more often the dark and light green areas are sharply differentiated. An arrest in the development of the midrib and principal lateral veins of the leaf occurs. Diseased leaves are distinctly smaller than healthy leaves, and often show the presence of blistered spots upon the blade. Finally, in the later growth of the plant, leaves may appear which are apparently normal, and these may be followed by normal flowers and fruits.

Heintzel (1900) studied the mosaic symptoms on *Nicotiana macrophylla* and *N. Tabacum*. The disease symptoms in *N. macrophylla*, consist in the appearance of dark green spots along the veins of the youngest leaves; these leaves become misshapen, their margins being bent under, and their tips elongated into beaks. In *N. Tabacum*, Heintzel found the symptoms quite different: leaf deformation was seldom noticeable; the leaf margins were seldom bent under, or the blade wrinkled; he never found dark green areas along the veins in this species; on the contrary the entire leaf blade was dark green with light green spots scattered over the entire surface so that it appeared mottled.

Iwanowski (1903) reports that the mosaic disease in *Nicotiana Tabacum* first becomes evident as a chlorosis of the leaves. He reports that often the dark green areas run along the veins only, but generally the two colors are intermingled irregularly. He also points out that the youngest leaves of the plant alone become diseased, and that the disease spreads from the point of inoculation to the growing point, affecting the new developing leaves.

Allard (1914a) finds that the character and intensity of the mosaic symptoms vary greatly, depending upon the age of the plants, rate of growth, and external conditions of the plants affected. He lists the symptoms which are characteristic of the disease in one or more of its phases as: (1) Partial or complete chlorosis; (2) Curling of the leaves; (3) Dwarfing and distortion of the leaves; (4) Blistered or 'savoyed' appearance of the leaves; (5) Mottling of the leaves with different shades of green; (6) Dwarfing of the entire plant; (7) Dwarfing and distortion of the blossoms; (8) Blotched and bleached corollas (in *N. Tabacum* only); (9) Mosaic sucker growths; (10) Death of the tissues (sometimes very marked in *N. rustica*).

Allard states that in plants approaching maturity, mottling in the upper leaves is the only sign of the disease. Actively growing plants may show fantastic ribbon leaves, and other irregular leaf outlines. Affected leaves often show abnormally thickened veins, reduction in area of the leaf blade, and the appearance of the striking, abnormal dark green blotches or patches which are often savoyed. Allard found that the flowers of *N. Tabacum* showed a distinct blotching or bleaching of the pink corollas. He also found distortion of the floral organs, the corollas misshapen, and containing within them partially developed and misshapen stamens and pistils, and small seed pods with shrunken seeds.

## 2. *Symptoms of other mosaic and related virus diseases of plants*

Dickson (1922) describes the mosaic symptoms of a number of plants affected with mosaic disease, including tobacco, potato, tomato, petunia, pepper, beans, raspberry, and others, and notes in a review of the symptoms in all these plants the similarity that exists.

Smith (1891, 1894) describes the symptoms of two different diseases of peach trees which are evidently related to the virus diseases. Peach yellows is characterized by a red spotting and abnormally early maturity of the fruit. A premature development of ordinary winter buds, or of buds buried in the bark of trunks and limbs of the trees, into slender, pale shoots, or into branched broom-like growths occurs. The duration of the disease varies greatly, the symptoms progressing slowly from limb to limb. In peach rosette the whole tree is usually diseased, and six months is enough to destroy the entire tree. The disease becomes evident upon the unfolding of the short axis buds where compact tufts of from two hundred to four hundred diminutive leaves with as many misshapen stipules are found. The leaves appear stiff, due to a peculiar straightening of the midrib. The trees drop their fruit very early while it is still green or yellowish green. The prevailing color of foliage is yellowish green or olivaceous. The compact bunching of the leaves is very conspicuous and makes the tree look quite different from one affected with yellows.

Chapman (1913) states that the leaves of mosaic diseased tomato plants are mottled, and appear stiff and badly curled.



The light green areas on the tomato fruits become yellowish, and in badly affected plants, purplish red.

Allard (1916b) describes a mosaic disease of petunia plants in which the leaves are distorted, curled, and wrinkled, and show irregular dark green areas along the veins. He also (1918c) describes pokeweed mosaic as bearing a close resemblance to that of tobacco. McClintock and Smith (1918) describe the spinach blight disease as a specific disease differing from the mosaic disease of cucumber and tobacco in that the spinach plants are eventually killed by the disease. Doolittle (1918) describes the cucumber mosaic as causing a mottling, savoying and wrinkling of the leaves, dwarfing of the stems and petioles and the appearance of dark and yellow green areas on the fruits. The dark green areas are raised to form wartlike protuberances.

Ensign (1919) describes a mosaic disease of sweet potatoes, in which the leaves are dwarfed, malformed, and mottled, and the yield of roots considerably reduced. Brandes (1919, 1923) describes the symptoms of the mosaic disease of sugar cane and other grasses as a mottling and striping of the leaves. In advanced stages of the disease, the plants are dwarfed, and yellowed, though seldom killed.

Gardner and Kendrick (1921, 1924) describe a mosaic of soy bean in which the leaves are stunted and misshapen, and show puckered, dark green, savoyed areas along the veins, and a mosaic disease of turnips in which the leaves appear lighter green with dark green blisters or puffy areas scattered over the blade. Jagger (1921) describes a transmissible disease of romaine lettuce which is first evident as a yellow discoloration along the veins of young expanding leaves. Schultz (1921) describes a transmissible disease of Chinese cabbage, mustard, and turnip, in which there is a distinct mottling of the leaves, a distorting and ruffling of the leaf blade, and a dwarfing of the plant. Lyons (1921) describes three major diseases of sugar cane. Yellow stripe disease and sugar cane mosaic are said to be identical. 'Sereh' is a disease of sugar cane apparent in the formation of bushy tufts of leaves from an arrested growth of the canes. The Fiji disease is a third disease of the sugar cane, characterized by the appearance of galls or swellings on the under surface of the leaves. Dickson (1922) describes mosaiced

raspberry plants as bearing leaves mottled with dark and light green areas varying in shape and size. The leaflets are small and frequently deformed. The dwarfing of the plant varies in degree from hardly any to the production of very severely dwarfed forms in which the stems are also spindling. Melhus (1922) finds that the mottling and crinkling that are so characteristic of mosaic diseased plants are masked in egg plant (*Solanum Melongena*) in the greenhouse in the case of plants that have passed the seedling stage. The only evidence of an abnormal condition is the smaller size and the infectiousness of the juices when inoculated into tomato, where they produce typical mosaic symptoms.

Rand (1922) describes the symptoms of pecan rosette as evident in an undersized and more or less crinkled leaf, which upon further development becomes mottled with chlorotic areas between the principal veins. The leaves are often malformed, with the leafblade suppressed, so that a midrib with merely a ragged edge of blade appears, a condition which suggests the frenching of tobacco leaves. Rankin and Hockey (1922) describe leaf curl or yellows of raspberry as differing from mosaic of raspberry. In leaf curl the leaflets appear darker green than normal, and the midvein arches downward. A similar arching of the lateral veins causes a downward curling of the entire margin of the leaflet.

McKinney (1923, 1925) describes a rosette and mosaic disease of wheat and rye, in which the mottling consists of irregular streaks in the long axis of the leaves, and in which the plants are often dwarfed and inclined to excessive tillering. Carsner and Stahl (1924) describe the curly-top disease of the sugar beet as evident in a curling of the leaves, and the appearance of irregular swellings of the veins on the under surface of affected leaves. Marked phloem necrosis is produced throughout the plant. Doolittle and Jones (1925) describe a mosaic disease of peas and sweet peas, in which the leaves are mottled, with numerous small dark green areas occurring along the veins. Zeller (1925) describes loganberry dwarf as a disease in which the internodes appear shortened, and the leaflets small and yellowed.

### 3. Range of hosts susceptible to mosaic and other virus diseases

Allard (1914a) found that the mosaic disease of tobacco is transmissible to many other species of *Nicotiana*. He states however that *N. glauca* and *N. glutinosa* were not affected. In a later report (1916c) he claims that a mosaic disease of *N. glutinosa* is distinct from that of tobacco, and that the only plant he found susceptible to both mosaic viruses was *Datura Stramonium*. Walker (1925) was able to transmit a mosaic disease from tobacco to *N. glutinosa* and from the latter to tobacco again, and also to tomato. He concluded therefore that there do not exist in *Nicotiana* two strains of tobacco mosaic as Allard claimed. Walker suggests that Allard ran his experiments during the winter months, when *N. glutinosa*, because of retarded growth, does not show the symptoms of disease in a very decided manner. Johnson (1926) suggests that both Allard and Walker were probably working with 'cucumber mosaic,' which Johnson has found will produce typical mosaic symptoms in *N. glutinosa*. He favors Allard's interpretation of the existence of two strains of virus.

Allard (1914a) was able to transmit the mosaic disease from tobacco to petunia, tomato, two species of *Physalis*, *Datura*, *Solanum nigrum*, and several species of *Capsicum*. He states that the mosaic disease of tobacco is distinct from that of pokeweed. Brandes (1919) found that more than a thousand varieties of sugar cane, including all the commercial varieties, are susceptible to sugar cane mosaic. Corn, sorghum, rice, millet, the crab grasses, fox tails, and *Panicum* were all found to be hosts of what appears to be an identical disease agent. Doolittle (1921) and Doolittle and Walker (1925) show that cucurbit mosaic is capable of transmission to a wide range of hosts, including milkweed, pepper, pigweed, wild cucumber, pokeweed, catnip, and all the species of Cucurbitaceae tested. Kunkel (1925) finds that aster yellows can be transmitted through insects to fifty different species of plants in twenty different families. The disease however shows great variation in the symptoms it produces in the different hosts.

Elmer (1925) finds that he can cross-inoculate mosaic disease between hosts belonging to distinct orders and families. Artificial inoculation appears to be more difficult and the incubation

period longer when the cross inoculations are made between families than when the inoculations are made within the same family. For successful cross inoculations he recommends that the plants to be inoculated and the plants furnishing the virus should all be growing vigorously.

#### 4. *Strains of mosaic virus*

The presence of two or more strains of virus native to a single plant, and even capable of producing distinctive sets of reactions within a given host, has been demonstrated in a number of cases. The most notable work on the existence of distinct strains of virus in the potato, and their isolation is that of Schultz and Folsom (1919, 1920, 1921a, 1923). They distinguish seven distinct and transmissible degenerative diseases of potato, each with its own characteristic symptoms in the growth habits of the plant, in effects on the structure of the tissues of the stem, and in chlorotic leaf patterns. These diseases may be transmitted singly or in various combinations to healthy potato plants. The strains of virus are characterized as: 'mild mosaic,' 'leaf rolling mosaic,' 'rugose mosaic,' 'streak disease,' 'leaf roll and net necrosis,' 'spindling tuber,' and 'unmottled curly dwarf.' All these diseases are believed to be due to distinct though similar viruses.

Goss and Peltier (1925) confirm the work of Schultz and Folsom as to the existence of distinct strains of virus in potato which run true to type by tuber perpetuation when the possibility of infection with other virus diseases the previous year has been eliminated. Johnson (1924) has found that ordinary mosaic virus can exist simultaneously with the 'mottle type' of virus obtained from 'healthy' potatoes. The two viruses, when introduced into healthy tobacco plants, together produce a marked necrosis in the plant which neither virus alone ever produces. Dickson (1925) finds that the combination of two mosaic viruses, namely that of potato and that of tomato or tobacco (these two mosaics being considered identical), when inoculated into tomato, produce in the tomato a characteristic disease symptom of streaking or striping of the tomato stems.

Fernow (1925) was able to distinguish eight distinct strains of mosaic virus. Johnson (1926) has found that tobacco plants are susceptible to at least five distinct strains of mosaic

virus: 'tobacco,' 'cucumber,' 'petunia,' 'speckled' and 'mild tobacco' mosaic viruses.

### 5. *Methods of disease transmission*

Although the mosaic and related diseases have been shown to be of an infectious nature in nearly every case, the natural methods of communication of the disease in the field, and the experimentally demonstrated methods of disease transmission are considerably varied. Mayer (1886) was the first to show the transmissibility of the tobacco mosaic disease. He pressed out juices of diseased plants, filled capillary tubes with them, stuck the tubes into healthy plants, and thus secured the disease in two or three weeks.

a. *Rubbing the plants with juices or stabbing them with a needle dipped in virus.* In tobacco the disease is transmitted easily from one plant to another by gently rubbing some juice of a diseased plant on a healthy plant, or by inoculation with a needle dipped in virus. In these cases a single inoculation is sufficient to bring about a virulent attack of the disease. The extreme opposite of this is found in Johnson's (1925) report of transmission of disease to healthy tobacco plants by the use of juices from healthy potato plants as the source of the inoculum, in which he finds that more than twenty inoculations are necessary to bring about infection.

Allard (1917) found that spraying or dropping the virus upon tobacco leaves had no effect. If the leaf is uninjured, no infection will take place. Rubbing the leaves with the virus, produces the infection by breaking the trichomes. Again he found more than one inoculation, or several inoculations at various points, more effective than a single inoculation.

b. *Transmission by grafting.* Smith (1891) found in the case of peach yellows and peach rosette, that even seemingly healthy buds from diseased trees, when grafted upon healthy trees, would produce the disease in these trees. Baur (1906, 1907) found that the infectious chloroses of certain variegated species of Malvaceae could not be transmitted by means of inoculations of juices obtained from the variegated plants, but only by means of grafting. Smith and Bonquet (1915) found that the curly top disease of sugar beet is transmissible by grafting buds connected with wedge shaped pieces of root tissues from diseased

beets into shoulders of healthy ones. McClintock (1923) verified Smith's claim that peach rosette is an infectious mosaic capable of transmission only by grafting buds of diseased plants upon healthy plants.

c. *Transmission by insect sting or feeding.* Shaw (1910) found that the leaf hopper *Eutettix tenella* introduces the active agent of the curly leaf disease into healthy beet plants. It seems possible also that some developmental stages of the virus are also gone through within the insect's body, since the insects are unable to communicate the disease until one or two days have elapsed from the time of feeding on infected plants. Allard (1914a) found that the aphid *Macrosiphum Tabaci* is a carrier of the mosaic disease of tobacco. McClintock and Smith (1918) found that the spinach blight could only be transmitted by means of aphids. They found that the causal agent of the disease could be transmitted by aphids to their offspring which in turn could produce spinach blight in healthy plants.

Brandes (1920) found that artificial inoculation of sugar cane mosaic was rather difficult, but he did succeed in communicating the disease to healthy plants in the greenhouse by hypodermic injections at the growing points of the expressed juice from diseased plants. The same author (1923) has described the mechanics of inoculation with sugar-cane mosaic by *Aphis maidis*, which may simply act as a vector or possibly as an intermediate host.

Severin (1922) found that the beet leafhopper *Eutettix tenella*, which carries the agent producing the curly leaf disease in beets, is non-virulent when it hatches from the egg. A minimum incubation period of the causative agent in the beet leafhopper is four hours at the following temperatures: maximum, 103° F; minimum, 94° F; mean, 100° F.

Wilcox and Smith (1924) found that the aphid, *Amphorophora rubi*, when transferred from healthy raspberry to mosaic diseased leaves of King red raspberry, and then placed upon young tip leaves of healthy Kansas black raspberry, could induce the mosaic symptoms.

Kunkel (1925) found that the leaf hopper *Cicadula sexnotata* is the specific carrier of aster yellows. Olitsky (1925b) found that the mealy bug *Pseudococcus citri*, can spread the mosaic disease of tobacco to healthy plants. Doolittle and Walker

(1925) found that three insects are instrumental in carrying cucurbit mosaic from its numerous wild hosts to the cultivated cucurbits; they are the cucumber aphid, the striped beetle, and the twelve-spotted beetle.

d. *Seed transmission.* It is generally accepted in the literature that tobacco mosaic is not transmissible through the seed. Allard (1915b) believes this is due to the fact that embryonic development never proceeds in those ovules actually invaded and infected by the virus. He asks the question, "What protects the embryo so securely from the mosaic disease even though associated with and nourished by infective parental tissue?" Quanjor (1920) suggests as an answer that if we suppose the causal agent of mosaic to be an ultra microscopic one, one can readily understand how the embryo would be protected from infection. The connection of the phloem strands with the embryo is twice broken, once between the mother plant and the endosperm, and the second time between the endosperm and the embryo. The embryo feeds by osmosis and can only absorb fluid matter.

For this theory one must accept what has not at all been demonstrated, that the virus travels through the phloem strands only; and second, that a living parasite could not of itself enter the embryo, but must necessarily be barred by a plasma membrane.

Reddick, Donald and Stewart (1919) found that the mosaic of beans is transmitted through the seed. Gardner and Kendrick (1922) grew 20,000 tomato plants obtained from seed of diseased fruits, and found no evidence that tomato mosaic is carried through the seed. Newhall (1923) states that lettuce mosaic is frequently transmitted through the seed. Brandes and Klaphaak (1923) in testing for seed transmission among a number of grass species susceptible to sugar cane mosaic, secured only negative results.

Doolittle and Walker (1925) report that the seeds of wild cucumber carry the mosaic virus, but those of cultivated cucumber give only doubtful results. Doolittle and Jones (1925) report that 1900 pea plants of several varieties of peas were grown to maturity from seed obtained from mosaic diseased plants. The plants were covered with cheese cloth cages to prevent insect contamination. None of these plants showed any evidence of mosaic.

e. *Soil transmission.* Beijerinck (1899) found that when healthy plants are put into pots containing soil in which the virus is present, the plants became diseased. He suggests the probability however that the roots may be injured in some way and enable the virus to enter. Allard (1914a) placed healthy plant material in soil in which diseased plants had been grown, but did not observe any sign of infection.

Quanjer (1920) reports that in leaf curl or curly dwarf of potato, infection passed through the soil, and that in a heavy clay soil only the very nearest neighboring plants will be infected, while in light sandy or peaty soil, the infection may pass on to the third, fourth, or fifth plant. Other investigators doubt this and believe the infections occurred through insect transmission, as Quanjer did not attempt to protect his plants from insects. This is the suggestion of Schultz and Folsom (1921) who obtained only negative results with soil transmission experiments.

Recently McKinney (1925) reports that the causal agent of wheat and rye mosaic may persist in the soil and cause infection of young plants. He finds the causal agent persisting in fine river soils for at least six years, and that susceptible varieties of wheat never fail to develop the disease when grown in such infested soils out of doors.

#### 6. *Carriers of mosaic*

Nishamura (1918) transmitted the mosaic disease of tobacco to *Solanum aculeatissimum* and to *Physalis Alkekengi*. He found that the latter did not show the symptoms when inoculated but that its juices now contained the infectious principle, which is able to induce the mosaic disease in tobacco when inoculated into that plant. This species of *Physalis* acts as a symptomless carrier of the mosaic virus of tobacco.

Carsner (1919) and Carsner and Stahl (1924) have found that the virus of the curly top disease of sugar beets can exist in certain plants, such as *Chenopodium murale* and *Rumex crispus* and others, without any external evidence of disease appearing in these plants. The virus, however, becomes attenuated, so that when injected into beets again it produces a milder phase of the disease. These plants evidently act as carriers of the curly top virus of beets.



Schultz (1925) has described the appearance of a form of necrosis or 'streak' disease of potatoes resulting from cross inoculations of juices between healthy potatoes. He suggests that these healthy potato plants were acting as carriers of the viruses, since they are themselves susceptible to ordinary streak disease of potato and showed no sign of the disease.

Johnson (1924, 1925) reported several new mosaic diseases in tobacco produced by inoculating healthy tobacco plants with the juices of what are apparently healthy potato plants or potato tubers. He has also suggested that these apparently healthy potato plants may be acting as carriers of several distinct potato viruses.

### 7. *Filtration studies*

With the continued failure to find a visible organism as the causal agent of the mosaic and allied diseases, investigators subjected the expressed juices to all sorts of experimental procedures in the hope that their results would show the true nature of the causal agent.

Iwanowski (1892) was the first to demonstrate that the causative agent of a disease could be passed through a bacterial filter, when he found that the juices from diseased tobacco plants after passage through the Chamberland bacterial filter, still retained their infectious properties. Beijerinck (1899) and Iwanowski (1903) agree that the passage of the virus through the filter seems to render the virus less virulent. The finer the pores of the filter, and the less the pressure, the weaker will be the infective qualities of the filtrate. Iwanowski believes that this suggests that the infective particles are actually being held back by mechanical means, and therefore the virus is not of the nature of a fluid.

That there is a limit to the possibilities of passage through bacterial filters by the virus is evident from Allard's (1916a) studies. He found that filtration of the virus of tobacco mosaic through a normal Berkefeld filter did not deprive the juices of their infective properties, but that there was evidence that the virus had become attenuated and less infectious. When the virus was filtered through a Livingston atmometer cup, the virus was completely gone from the filtrate.

Duggar and Armstrong (1923) reported that it was possible

to find a filter which, in a given interval of time, at a given pressure, permitted only a relatively small number of the infectious particles to pass through. A standardization of the filters was accomplished by testing their capacity to permit or prevent the passage of colloidal particles of known or approximately known sizes to pass through. It was found that the infective particles of mosaic disease approximate in size those of a fresh 1 per cent haemoglobin solution, the particles of which are  $30\ \mu$  in size. This was shown when the 1 per cent solution of haemoglobin particles passed easily through the two filters through which the tobacco mosaic particles also passed easily, but did not pass through any of the rest, as was also true for the tobacco mosaic particles.

#### 8. *Properties of the virus*

Beijerinck (1899) found that heating the virus to the boiling point killed it. The critical temperature lies between  $70^{\circ}$  and  $80^{\circ}$  C. At  $90^{\circ}$  C. the virus loses its virulence. Dried mosaic leaves preserved in the herbarium retained their infectious properties for two years. An alcohol precipitate from the expressed juices of diseased plants retains its virulency after dessication at  $40^{\circ}$  C. The contagious stuff can conserve its virulency in dry soil through an entire winter.

Allard (1914a) also found that the causative agent showed a high degree of resistance to the ordinary destructive agencies. He (1916a) found that the infective properties are destroyed by the higher alcohols, an 80 percent solution of ethyl alcohol destroying the infectious nature of the virus in half an hour. Hydrogen peroxide will destroy the peroxidase in the virus without destroying the infectious particles, but a concentration point may be reached where the excess of peroxide also kills the infective particles. Ether, chloroform, carbon tetrachloride, toluene, and acetone do not extract the infective material or destroy it. Allard (1918) found that mercuric chloride affected the virus very little, but that copper sulphate was rather toxic. When mixed with talc, kaolin, or soil, the virus loses its properties more quickly than when bottled without any preservative at all. Allard (1914a) found that the bottled sap of diseased plants after undergoing fermentation was able to produce infection four or five months later.

Dickson (1925) used the unfiltered expressed juices of mosaic diseased plants preserved in a small bottle since 1920, and only protected from contamination by a layer of toluene, to inoculate plants in 1925. He found the juices still retained their infectious properties, since all the plants inoculated became diseased.

Duggar and Armstrong (1923) found that the virus of tobacco mosaic resisted dehydration with acetone and alcohol. It could not however withstand complete dehydration. When compared with the spores of *Bacillus subtilis* the virus is less resistant to dehydration. The authors studied also the effects of grinding on the infectivity of the tobacco virus. They found the virus highly resistant to protracted grinding.

Walker (1926) found that the infective principle contained in the expressed juices from mosaic diseased tomato and ground cherry plants can resist ageing, drying, heat, alcohol, dilution, and filtration in the same way that the tobacco virus has been shown to do, but that the virus of cucumber is much less resistant.

#### 9. Attenuation of the virus

Beijerinck (1899) secured an attenuation of the virus of tobacco mosaic. By mixing the virus with a culture of *Bacillus angulomerans* (an organism isolated from mosaic diseased tobacco plants) a new symptom was produced in the leaves, in which the leaves were spotted with light green blotches showing complete loss of chlorophyll, and the leaves looked very much like types found in decorative variegated plants. Inoculation of plants with virus mixed with formalin did not produce the disease in the plants, but instead this same sort of variegation. When the virus was allowed to remain a long time with the formalin it produced no effects upon the plant at all.

Allard (1915a) in testing the effect of dilution upon the infectivity of the virus, found that dilutions of one part of virus in one thousand parts of water were just as effective in producing the disease as the original undiluted virus. One part of the virus in ten thousand parts of water gave evidence of attenuation. A dilution of the virus of one part in one million parts of water gave no infection at all.

Carsner and Stahl (1924) and Carsner (1925) found that the passage of the virus of curly top disease of sugar beet through the nettle-leaved goosefoot, *Chenopodium murale*, or through

*Rumex crispus*, and *Suaeda Moquini*, attenuated the virus to such a degree that when transmitted to healthy sugar beets it produced only a mild phase of the curly top disease in those plants.

10. *Effects of environment on the expression of mosaic symptoms*

Lodewijks (1910) experimented with different colored lights upon the expression of mosaic symptoms in tobacco plants affected with the mosaic disease. When the upper part of a diseased plant was covered with a red jar, the spots on the diseased leaves tended to spread out and become less noticeable. The youngest leaves showed only a few light spots, and the new leaves were pure green. When the upper diseased portion of the plant was covered with a blue jar, recovery took place in 15 days, new leaves and old leaves appearing pure green. His explanation of this is that the formation of virus (toxin) is inhibited in the upper leaves by the weakening of the illumination, while the healthy lower leaves can build anti-virus to combat the virus in the upper diseased regions of the stem. Freiberg (1917) regards Lodewijk's results as simply due to shading of the plants. The cutting off of the light thus reduced metabolic activity and growth, and so the symptoms of the disease were masked to a greater or less degree.

Johnson (1922) found that the optimum temperature for the expression of mosaic disease symptoms in the potato lies between 14° and 18° C., and that above 20° C. the symptoms disappear. A temperature of 24° to 25° is necessary to completely inhibit the disease. Tompkins (1925) found that relatively short exposures to high temperatures are sufficient to completely mask mosaic symptoms in potato.

Goss and Peltier (1925) also found that temperature has a pronounced effect upon the masking of mosaic symptoms as expressed in the foliage. The mottling of 'mild mosaic' of the potato disappears entirely above 25° C. from young developing shoots, and the symptoms are often so masked that the plants appear healthy. The 'rugose mosaic' symptom of diffuse mottling was not changed by high temperatures. It was found that plants affected with 'spindling tuber' showed an increase in the severity of the symptoms of ruffling and uprightness at higher temperatures, but masking of the symptoms at lower temperatures (the reverse of 'mild mosaic').

### 11. *Culture of the supposed organism*

Olitsky (1924, 1925a) reported that he had grown the active agent of tobacco mosaic in culture. The medium employed was an aqueous extract from healthy leaves and stems of tomato plants. Staining and dark field illumination of the material of the cultures showed merely a greater number of granules in the mosaiced cultures than in the control. Since in the successive subplants a dilution of the virus had been reached far beyond that of 1 to 1,000,000, Olitsky assumed that multiplication of the virus must have taken place, as otherwise the final material could not have been infectious. Mulvania (1925) and Purdy (1926) report the repetition in every detail of Olitsky's experiments, but do not confirm his results. They found no indication of an increase in the virus in their successive transfers to new subplants.

### 12. *Nature of the causal agent*

Beijerinck (1899) decided that the causal agent of the virus diseases of tobacco must be in the nature of a contagious solution—a *contagium vivum fluidum*. He came to this conclusion upon finding that the infectious material of diseased leaves was able to diffuse downward through agar plates. He decided that since the virus was able to diffuse through the agar, it must be in the nature of a liquid, and water soluble, rather than corpuscular. From the fact that a very minute quantity of the virus, when introduced into a plant, will produce the disease, and that from this diseased plant one can now obtain an indefinitely large quantity of virus with which to inoculate other plants, Beijerinck concluded that the virus must multiply within the inoculated plant. It thus appeared to him that the causal agent must be of the nature of a living contagious liquid.

Woods (1899, 1900) attributes the mosaic disease to an excessive accumulation of oxidizing enzymes in the tobacco plants, so that they are unable to develop a normal amount of chlorophyll, and hence appear badly nourished. He suggests that under certain conditions, such as partial starvation brought on by fungus, bacterial, or insect attack, or by unsuitable conditions of soil nourishment, the enzymes in the plant become more active or else are produced in abnormally larger quantities. Since chlorophyll is converted by oxidizing enzymes into xantho-

phyll, an abnormal quantity of enzyme in the plant tissue will produce the variegations in the coloring of the leaves such as are present in ornamental plants and in the leaves of mosaic diseased plants. He reports that the peroxidases were twice as strong in the light colored areas as in the green areas of diseased leaves or in healthy leaves.

Iwanowski (1903) concluded that the causal agent of the tobacco mosaic disease is bacterial. Although he found amoeboid bodies in contact with the nuclei of the cells in the diseased areas of the leaves, he suggests that they must be products of amitotic nuclear division or reaction products of the cells to the disease, and cannot be the causal organisms of the disease, since bodies as large as these could not possibly pass through a bacterial filter. Stained sections revealed the presence of bacteria lying in zoogloea-like masses in the cytoplasm. These bacteria were in the form of very minute, short rods. He was unable however to successfully culture a bacterial organism which would produce the mosaic disease.

Hunger (1905a, b) regards the mosaic disease of tobacco as having the nature of a *Stoffwechselkrankheit* in which the causal agent of the disease is not a living organism but of the nature of a toxin. This toxin, phytotoxin, can arise autonomously in the cell and be carried over into other plants. The toxin is distinctly localized because some localized factor has disturbed the metabolic activity and the assimilation of the particular cells in which it appears. Its effect upon other cells is to cause the cells to manufacture more toxin like itself.

Allard (1916a) states that all the evidence at hand in regard to the mosaic disease of tobacco indicates that the causal agent is an ultramicroscopic parasite of some kind. He disputes Woods' claim that it is the excessive oxidizing enzymes present in diseased tissues which produce the disease. When the juices of diseased plants are passed through the Livingston atmometer cup, the virus is entirely removed from the filtrate, since inoculation experiments with the filtrate show that it has lost all its infectious properties. However the filtrate still shows an intense peroxidase reaction.

Palm (1922) observed the presence of two kinds of foreign elements in mosaic diseased tissue of tobacco plants. Large amoebiform, or spherical bodies were frequently found lying

in intimate contact with the nucleus of a cell or in its vicinity. The bodies did not appear to have any definite structure, although one or more hollows resembling vacuoles could be made out, and when well stained, the presence of one or more granules within them. In cells containing these bodies, minute granules were also found. The granules lay in irregular conglomerations in the cell lumen, and often completely filled it. Palm concluded that the irregularly shaped corpuscles are only reaction products of the disease. The minute granules are considered homologous with the so called corpuscles of Garnier, which are found in the affected tissues of small pox patients, and associated with other virus diseases of man and animals.

Freiberg (1917) concluded that the properties of the infective principle of mosaic substantiates the view that the infectious substance is an enzyme and not a virus. This conclusion is reached because the various experiments of Allard and others have simply shown characteristics of the virus that may be true also of enzymes, as for example: the temperatures which destroy the virus also are those which inactivate enzymes; the infectious properties of the virus are destroyed by the same concentrations of alcohol which destroy enzymes.

McWhorter (1922) describes the presence of amoeboid bodies in the cells of galls caused by the Fiji disease of sugar cane. He believes these represent living organisms. He was able to cultivate them in hanging drop cultures. They reproduce in two ways: (1) by a simple constriction into two; and (2) by gemmation or the constriction off of small parts of the original body each containing a chromidial fragment of the original body nucleus. When the host cell walls of the gall begin to thicken, the bodies cease to divide, round up, and become encysted.

McKinney, Eckerson, and Webb (1923) describe the occurrence of intracellular bodies associated with wheat rosette and mosaic disease. They conclude the bodies are not artifacts, but are uncertain as to whether they represent living organisms or are formed by the reactions of the cells to the disease. The intracellular bodies are present in the crown tissue of winter wheat in late winter and early spring. As the disease progresses, the bodies become distributed throughout the entire plant and occur in the roots, crown tissue, leaf sheaths, and leaves. They

vary in form, being round to oval, irregular, or very long. They seem to increase in size with the age of the cell. Minute bodies are found in the very young cells of the central and lateral buds. These increase in size until large bodies are found in the oldest cells of the leaf sheaths and crowns. The bodies are homogeneous in content, and contain large and small vacuoles. In fixed material, they appear to be bounded by a membrane. The vacuoles often are surrounded by a densely staining ring and contain granule-like or elongated bodies, the structure of the vacuole suggesting a nucleus. The bodies did not appear to show any independent movement, but moved from place to place with the streaming cytoplasm.

Smith (1924) found amoeboid bodies in the cells of mosaic infected potato leaves. He suggests that these bodies are degenerated products of the nucleus of the cell, effects of the virus rather than the cause of the disease.

Rawlins and Johnson (1925) describe the presence of amoeboid intracellular bodies in the cells of diseased tobacco leaves. They studied leaves showing different stages in the development of the disease, but did not come to any conclusion as to the existence of a possible cycle or evolution of the various sized bodies and various shaped forms they found in the diseased tissues. They found that the presence of the bodies in young dividing cells does not hinder or distort the cell division. They saw no evidence that the bodies divide or reproduce. They found that the vacuolate bodies often contained dark staining granules in their vacuoles which suggest the structure of nuclei found in some *Amoebae*.

Kunkel (1925) is of the opinion that the intracellular bodies associated with so many of the virus diseases must be of the nature of living organisms. The amoeboid form found in the tissues may represent perhaps only one stage in the life of the causal agent. He suggests that at one time they may be very minute and plastic enough to pass through bacterial filters, and only become visible after a certain period of growth within the host cell. In corn he reports (1921) that the bodies are only found in diseased tissues and never in the dark green parts or in healthy plants. Their protoplasmic appearance, their staining reactions, their amoeboid shape, and position with reference to the nucleus of the host cell, suggest that they are probably



living organisms. He does not believe that waste products in a cell would show these characteristics. In connection with a study of the Fiji disease of sugar cane, Kunkel (1924a) again states that the bodies associated with this disease appear to be living organisms, in that they grow and divide, and are found only in the tissues of the gall and spread with its growth.

Eckerson (1926) describes the appearance of motile organisms in the form of tiny flagellates in tomato after inoculation with tomato mosaic virus.

## II. A DEVELOPMENTAL STUDY OF THE LEAF SYMPTOMS AS EXPRESSED IN THE LEAF PATTERNS OF DISEASED LEAVES

I have undertaken a more intensive study of the symptoms of tobacco mosaic with the hope of adding to our knowledge of the effects of the causal agent, whatever it is, on the structure and functions of the diseased cells. Such knowledge is necessary if we are to understand the relations of the filterable virus diseases to the phenomena of variagation, physiological chlorosis, etc., of which the effects in leaf coloration are so similar in many cases to those of true mosaic. Further such data are necessary as a basis for more accurately judging the evidence for the existence of specific strains or races of mosaic viruses. As is well known, potato mosaic exists in a considerable series of distinguishable forms. I have found some evidence for the existence of a second strain of tobacco mosaic. For the adequate differentiation of such variants, full knowledge of the visible effects they produce in the diseased cells is essential.

In my experimental work with the mosaic disease of tobacco, I have used only plants of *Nicotiana Tabacum* grown from commercial seed of the variety known as Connecticut Seed Leaf (Connecticut Broadleaf Tobacco). These plants take the disease very easily, and have shown very uniform growth and inherited vigor when grown under identical conditions of culture, and continuous repotting. I have aimed at an intensive study of a single strain as preliminary to a comparative study which must include other strains and species.

Plants of this strain which are of uniform age and under uniform cultural conditions, when each is inoculated in the corresponding leaf and with virus obtained from a single diseased leaf, show a remarkable degree of uniformity in the leaf pattern

symptoms. This is true, not only for a single stage, but for all the successive stages in the development of the disease. So far as I can find from the literature, no one has described as such the developmental series of leaf patterns peculiar to tobacco mosaic. The symptoms listed below have all been noted, but not as occurring in any definite sequence or at any definite period after inoculation.

The succession of leaf patterns described here has been found to occur repeatedly when the plants are grown under uniform conditions and inoculated by uniform methods in the corresponding leaves. I have kept a record of the position of each leaf and its size, with the particular symptom pattern which appears upon it first after inoculation, and of the succession of patterns which occur later on it and on the other leaves of the same plant. I have also studied cytologically the modifications of structure in such leaves.

Although in a given series of plants we may have any one pattern type present in a varying number of leaves, nevertheless there is a distinct correlation evident between the particular pattern type exhibited by a leaf and the following conditions: first—whether the leaf was fully formed, in an embryonic condition, or not yet started at the time of the inoculation; second—the position of the leaf with respect to what I shall call the critical leaf (the leaf nearest to the inoculated leaf which on the appearance of the disease shows the most pronounced symptoms); third—the position of the leaf with respect to the base of the plant, that is whether it occurs in the region of the stem of less or greater elongation; fourth—the length of time the disease has been evident in the leaf. My experience with the variety of tobacco I have used, namely Connecticut Seed Leaf, and with the strain of virus I have had, shows that, in this case at least, a distinctive series of leaf patterns will regularly be found.

#### *Classification of pattern types*

I have been able to distinguish and photograph a series of specific disease patterns and have found that the occurrence of each is correlated with the size and age of the leaf at the time of the entrance of the virus into it. The pattern itself is brought about by the specific effect of the virus upon the cells at each successive stage in the histogenic development of the stem and

leaf tissues. I am naming tentatively and presenting a brief descriptive outline of the six pattern types that I find, and the cytological structure of which these patterns are the visible expression.

*Type 1: Dark, vaguely blotched* (PLATE 19). Type 1 is a pattern characteristic of large, well developed leaves, and evident only in a rather even mottling, consisting of paler green rounded areas in the general dark green of the leaf blade. Cytological study shows that the leaf has attained to mature anatomical structure, and that this type of mottling is due to the effects of the virus upon the cell contents. All the cells in the lighter areas present about the same appearance as to the structure of the nuclei, the density and distribution of the cytoplasm, and the presence of the striated bodies and x-bodies. The difference in color must arise from the effects of the disease upon the chlorophyll and the plastids. The paler color found in the blade of the living leaf is evident in fixed sections only in the different staining reaction of the starch in the plastids of the two regions. The starch of the plastids of the lighter green regions stains red or reddish blue, while that of the darker regions stains blue.

*Type 2a: Pale crinkled* (PLATE 20, FIG. 1). Pattern type 2a is characteristic of leaves which have not attained their full size at the time of inoculation, and continue to grow during the incubation period. This pattern is characterized by the pale green color of the leaf, which is retained throughout the life of the leaf, and the appearance of faint lighter green spots or areas (pale mottling) in the centres of the vein islets. The veins are decidedly prominent, and appear whitish in color. The blade is also markedly crinkled.

*Type 2b: Pale vaguely blotched* (PLATE 20, FIG. 2). A leaf showing pattern type 2a (pale crinkled), upon further expansion, presents a more flattened blade, which is still very light green in color and shows the earlier rather vague mottling a little more definitely. This later and older phase of the disease pattern is designated as type 2b. Both leaf patterns (types 2a and 2b) show upon sectioning a more or less uniform type of anatomical structure throughout the blade. The cells are smaller than those in type 1 (dark, vaguely blotched) since these leaves are in reality younger than those showing type 1. Again

the vague mottling is evident in sections only by the staining reactions of the plastids, those in darker green areas staining more blue than those in the lighter areas, which stain with a slight bluish red tinge. All the cells show the presence of striated bodies, x-bodies, and other signs of disease, such as the presence of thick bridges of cytoplasm lying across the single vacuole of the cell, and enlarged nuclei.

*Type 3: Narrow nervisequum* (PLATE 21, FIG. 1). Type 3 is a pattern, shown by leaves which develop immediately after the initial appearance of the disease symptoms described as types 1 and 2a, on young leaves present on the plant during the incubation period. In this pattern we find narrow bands of very deep green along the smaller veins only. These bands are more or less uniformly scattered over the blade, which is itself in ground color a very light shade of green. The cells of the dark green areas alone contain normal plastids. More generally the dark green areas show an anatomical structure very different from that of the light areas. There are two layers of palisade cells in them, and the blade is seven layers thick, as it is in a healthy mature leaf. No striated bodies or x-bodies occur in these dark green areas. The cells contain healthy plastids, and these usually larger than they are in corresponding cells of healthy leaves. The greater part of the leaf blade, which is very light green in color, shows only six layers of cells, such as are found in very young leaves, no differentiation of palisade cells, and the cells are all cuboidal in form in all the layers. Although retaining the embryonic cell form, the cells show the content of mature cells. There is a single central vacuole, and the few plastids lie in the primordial utricle about the walls. The cells contain numerous striated bodies and x-bodies.

*Type 4: Malformed, broad nervisequum* (PLATE 21, FIGS. 2, 3). The leaves that develop from the young primordia arising on the growing point after the initial appearance of the disease symptoms exhibit pattern type 4. Type 4 shows dark green areas in wide bands along the more important secondary veins of the leaf, while the portions of the blade between them are very light green in color. These leaves, since they have contained the virus from their early primordium stage at the growing point, show the most marked effects of the disease. The leaf blade

is contorted and ruffled, and its margins usually curled under. The margins are also lobed or crenately toothed, due to the inhibition of cell divisions at various points during the meristematic marginal growth of the young leaf.

The cells in the light green areas show the original cuboidal form, while those in the dark green areas show all stages of histogenic development, according to the size and the age of the leaf at the time the sections are made. At a very early stage, the dark green portions themselves may show only the cuboidal cells or a single palisade layer of scarcely elongated cells. A young leaf will show only a single palisade layer of slightly elongated cells. A somewhat older leaf will show a single palisade layer with perhaps the third layer of cells slightly elongated, but no more so than is the case in a leaf of the same age upon a healthy plant. A somewhat larger leaf will show two palisade layers, but the cells of both these layers are in reality shorter than those in a healthy leaf of the same size, and much narrower. In these leaves, however, the dark bands remain in this late histogenic stage of differentiation.

*Type 5: Pale, definitely blotched* (PLATE 22, FIG. 1). In pattern type 5, we have a blotched appearance of the leaf. The blade is a very light green in color, with slightly darker green round blotches scattered over it. Sections show that the cells of the different regions are practically alike, with a feebly developed single layer of palisade cells. The blotched appearance is wholly due to changes in the cell contents, there being more plastids and starch grains, and fewer striated bodies, in the darker spots of the leaf than in the lighter areas.

*Type 6: Irregular, narrow nervisequum* (PLATE 22, FIG. 2). In pattern type 6, the leaf is of a very light green color, with narrow green bands irregularly scattered along the smaller veins. The pattern and the histogenic structure of the light and dark green regions resemble those of type 3 (narrow nervisequum). The light green areas show six layers of cuboidal cells, while the dark green bands are made up of seven layers of cells, including two palisade layers.

#### *The development of the leaf patterns*

When a plant is inoculated in a leaf that is still actively growing, the mosaic symptoms may appear in all the younger

leaves above this leaf, and continue to appear with more or less virulency in all the new leaves formed upon that plant. In the earliest stages of the disease, the symptoms are first clearly pronounced in a leaf which is always above the inoculated leaf. Other leaves between it and the inoculated leaf may show disease symptoms too but of a less definite type. I shall refer to this leaf as the critical leaf, that is, the leaf which presents the first decided symptoms of the disease, and is nearest to the inoculated leaf.

Figures 1-4, plate 18, illustrate the comparative sizes of leaves on a plant which, upon inoculation with the virus, and the initial appearance of the disease, will show the various pattern types previously outlined, and which are at certain stages of histogenic development to be explained later in a discussion of the histogenesis of healthy and diseased leaves. Figure 1 represents the leaf which will show type 1 (dark, vaguely blotched) pattern upon evidence of the disease in the plant. Figures 2 and 3 are of leaves which will probably show type 2a pattern (pale crinkled). Figure 4 illustrates the size of the leaf, which, together with even smaller leaves at the growing point, will upon further expansion during the initial appearance of the disease symptoms show at first extreme crinkling, like leaves of type 2a pattern, but will then show the characteristic narrow green bands along the finer veins of the blade characteristic of type 3 pattern. The leaves which develop from the growing point itself, after the presence of the virus in the plant, will show the later patterns.

The leaves below the critical leaf and above the inoculated leaf may show faint but characteristic mottled effects. Such leaves exhibit the pattern which I have designated as type 1 (dark, vaguely blotched). Plate 19 is a photograph of a leaf of this type from a plant about one and a half feet high, in a twelve inch pot, and showing active growth, although diseased. The photograph was made with the aid of a yellow color screen, and transmitted light to bring out more distinctly the vague blotches characteristic of this pattern.

Leaves showing type 1 pattern are those which have either reached maturity at the time of inoculation, or during the incubation period, and are capable of very little further growth. As cytological study shows, their histogenic development is

complete and they show the anatomical structure of an adult leaf. The type 1 pattern is not found in plants inoculated in the tip of the plant when the inoculated leaf and the leaves below it are capable of considerable enlargement. As the photograph of plate 19 shows, this pattern is characterized by large blotches that are indefinite in outline, and scarcely paler than the surrounding areas. There may be a slight curling or ruffling present in the leaf blade. Such a leaf is 'dark cress green' in color, with blotches that are scarcely paler than the 'dark cress green' and barely darker than 'cress green' of Ridgway.<sup>2</sup> The blotches are scattered more or less uniformly over the entire leaf. Such leaves may appear only doubtfully or vaguely mosaiced at the time when the disease makes its appearance in the critical leaf, but in a few days show the characteristic symptoms of type 1 as described. If many leaves intervene between the inoculated leaf and the critical leaf, those leaves nearer the critical leaf will change from doubtfully mosaiced to decidedly mosaiced sooner than those nearer the inoculated leaf.

The critical leaf on a plant about a foot high, and growing vigorously (my plants at this stage are as a rule in twelve inch pots) always shows a definite leaf pattern that I characterize as type 2a (pale crinkled). This type of pattern is developed in leaves capable of growth not only at the time of the inoculation of the plant but also during the incubation period, and after the initial appearance of the disease symptoms. In transmitted light the leaf appears mottled because of the presence of slightly lighter regions in the center of each vein islet. Moreover each minor vein islet is raised or elevated in the center, giving the leaf as a whole a decidedly savoyed appearance. The veins appear very light and prominent in the depressions bounding the vein islets. Such a leaf is typically 'cress green' in color and, except when viewed by transmitted light, the shades of green can barely be differentiated. Figure 1 of plate 20 is a photograph of a leaf of this type from a vigorously growing plant, taken with transmitted light with the aid of a yellow color screen.

A leaf of this type 2a (pale crinkled) is still capable of further

<sup>2</sup> The colors found in the leaf patterns have been named by comparison with *Color standards and nomenclature* by R. Ridgway, Washington, 1912. A list of the shades found in the leaf patterns and the percentages of green and yellow they contain will be found in TABLE 4 at the end of this section (see page 533).

growth and expansion. Type 2b (pale, vaguely blotched) develops directly from type 2a. Here the crinkled effect of the blade is considerably lost in the expansion of it, but the leaf never becomes entirely flattened out, as is clearly evident in the photograph of this type shown in plate 20, figure 2. The veins are still prominent, and the blade is in general of a 'light cress green' color. Leaves showing type 2a (pale crinkled) or 2b pattern (pale, vaguely blotched) in addition to the barely perceptible mottling, may show also a few 'dark dull yellow green' spots or blotches scattered irregularly over the leaf blade. These spots may increase in number, and become very much more prominent with the further growth of the leaf. They may also later even disappear or become very inconspicuous, in consequence of a loss of green color in them or of an increase of green color in the leaf blade in general.

In the initial stages of the disease, the leaves above the critical leaf show the type 2a pattern (pale crinkled) in a less pronounced degree than the critical leaf. As the disease develops only a few of these (those immediately above the critical leaf) will develop the 2b pattern upon the expansion of the leaf. The youngest leaves develop a new pattern type which I have referred to as type 3 (narrow nervisequum). The early crinkled effect is lost entirely, and the leaf appears smooth, and of an even 'cress green' color. Scattered regularly over the leaf there appear 'dark dull yellow green' blotches in the form of short narrow bands along the small veins. The dark green blotches are present most often along both sides of the veins, and often become bullate or savoyed. In plate 21, figure 1, is shown a photograph of a leaf of type 3 pattern taken with transmitted light.

The leaves which appear later on the diseased plant, which were present as leaf primordia during the initial stages of the disease, show patterns of a type which mark the climax of the disease. All the symptoms are intensified, and include conspicuous mottling, rolling, waviness of margin or ruffling, crenation or lobing of the leaf margin, marked savoying, and many other deformities in leaf structure, such as absence of a portion of the blade, the entire blade on one side of the midrib, or the entire leaf blade on both sides of the midrib (frenching). This condition, type 4 (malformed, broad nervisequum), is



found in young leaves which develop from the growing point immediately after the appearance of the disease symptoms in the plant. The blotching consists of large 'dark dull yellow green' areas bullate or sunken, along the important secondary veins coming from the midrib. The blade between these deep green areas is 'light cress green' in color, and here and there smaller rounded 'dark dull yellow green' blotches may occur. The leaf blade generally remains small and shows all degrees of malformation.

Plate 21 shows photographs of leaves of type 4 pattern. Figure 3 is a typical leaf of this pattern produced upon a vigorously growing plant in a twelve inch pot, and shows the wide dark green bands that run along both sides of the secondary veins. Light green areas lie between the bands, and the remainder of the leaf where the dark green does not occur is also light green. The entire margin of the leaf is curled under, and the crenate edge of the leaf blade, and the bullate character of the dark green areas are very clear. This photograph, and also that of figure 2, showing a similar pattern in a very young leaf, when the dark green areas are only feebly developed, were taken with transmitted light and the aid of a yellow color screen.

The leaves which appear still later show type 5 pattern (pale, definitely blotched). In this pattern the leaf is 'light cress green' in color, and shows large slightly darker blotches. Very often types 4 and 5 occur together in different portions of the same leaf. Plate 22, figure 1 shows a photograph of a leaf of type 5 pattern. The leaf was photographed with transmitted light and shows type 4 (malformed, broad nervisequum) pattern in the upper third of the leaf, and type 5 (pale, definitely blotched) in the lower two-thirds of the leaf. The vaguely outlined blotches of this type are quite evident in the photograph, as well as the rolled margin of the leaf blade.

During the remaining growth of the plant, the new leaves appearing show type 6 pattern (irregular, narrow nervisequum). In type 6 the leaves are of a somewhat darker green ('parrot green') upon which are scattered irregularly along the smaller veins 'dark dull yellow green' blotches, often depressed and savoyed. This type resembles type 3 pattern (narrow nervisequum) and may often persist until the flowers appear. The blotches found along the veins often are not definite narrow

bands, but fade out into the vein islets and show no sharp boundary. Plate 22, figure 2 is a photograph of a leaf showing type 6 pattern in which the irregularly scattered bands of dark green are definitely bounded. The leaf is from a vigorously growing plant in a twelve inch pot, and the photograph was taken with transmitted light and the yellow color screen.

This series of mosaic patterns may be modified by the age, vigor, and cultural conditions of the plants in various ways. Very young plants with about six or more leaves when diseased rarely show the series of symptoms described for larger and more vigorously growing plants. This is because all the leaves are still capable of extensive growth at the time of the inoculation. Type 1 (dark, vaguely blotched) is never found, and type 2a (pale crinkled) rarely, save perhaps in the largest leaves, upon the young plant. Type 4 (malformed, broad nervisequum) usually appears at once in the new leaves developing from the growing point, and the leaf symptoms that follow are of the severest type, including extreme frenching and blistering.

When two patterns occur together in a leaf, they are such patterns as one would naturally expect to appear together, that is, they belong together or follow one another in the development of the patterns. Types 4 and 5 occur most often together. In such leaves, type 4 pattern (malformed, broad nervisequum) is often at the tip of the leaf or in the upper third of the leaf, while type 5 pattern (pale, definitely blotched) is in the lower portion of the leaf. In the same way patterns 5 (pale, definitely blotched) and 6 (irregular, narrow nervisequum) occur together.

Vigorously growing plants may show fewer leaves of type 2 pattern and perhaps none of type 3. In a severely diseased plant, type 3 (narrow nervisequum) does not occur at all, and type 4 (malformed, broad nervisequum) may occur in several leaves, rather than in one or two, as is usual. However, no matter how many leaves there are of each pattern, or whether any of these pattern types are omitted, the sequence of the pattern types that are exhibited remains the same. The correlation between age and capabilities for expansion of the leaf, and the mosaic pattern developed is maintained.

The evidence for the correlation between histogenic development at the time of infection and the type of leaf pattern de-

veloped is based on more than ten successive series of ten plants each, grown throughout the year, which were all intensively studied, as well as on many more series of plants upon which casual observations only were made.

A series, whose data are given in TABLE I, consisted of a set of ten plants all of the same age, vigor, and cultural conditions of growth. The plants were two months old, growing vigorously and had been repotted into twelve inch pots a few days before their inoculation, June 13. They finally attained a height of five feet in spite of their diseased condition. Each plant was inoculated in the tenth leaf from the base of the plant with virus obtained from a single diseased leaf, and in each case the incubation period was six days, evidence of the disease appearing as types 1 and 2 on June 19.

In each case the critical leaf showed type 2a pattern (pale crinkled), but the critical leaf was not in all cases at the same interval above the inoculated leaf. In four plants—3b, 3h, 3i, and 3j—the critical leaf was the seventeenth leaf on the plant, leaving a gap of seven leaves between the inoculated leaf and itself. In three plants—3e, 3f, and 3g—the critical leaf was the fifteenth leaf on the plant, leaving a gap of only five leaves from the inoculated leaf. In two plants—3c and 3d—it was the sixteenth leaf, and in one case—3a—the eighteenth leaf from the base of the plant.

As shown in the table, the leaves between the critical leaf and the inoculated leaf showed the type 1 pattern (dark, vaguely blotched), while those above the critical leaf showed type 2a in the early stages of the disease. The youngest of the leaves showing type 2a pattern (pale crinkled) later developed type 3 (narrow nervisequum) upon the appearance of dark green narrow bands along the small veins with the further growth of the leaf. Those leaves which developed during the last days, from June 27 to July 8, showed pattern types 4, 5, and 6.

In such a set of plants, all of the same age, vigor, and growth, all inoculated in a definite and corresponding leaf of the plant, with the identical virus obtained from the same diseased leaf, the pattern types and degree of virulency of the disease symptoms will correspond all through the series in their initial appearance and subsequent development. The leaves on the plants at the time of the inoculation, and the leaves which appear later

TABLE I

*A record of the successive appearance of the mosaic disease patterns in ten plants*

PLANT INOCULATED		3a	3b	3c	3d	3e	3f	3g	3h	3i	3j
Leaf	Date										
13	June 19					I					
	20					I					
	21					I					
	23					I					
	27					I					
	July 8					I					
14	June 19					I					
	20			I		I	I	I			
	21			I		I	I	I			
	23	I?		I		I	I	I	I		
	27	I?	I?	I	I?	I	I	I	I		
	July 8	I?	I?	I	I?	I	I	I	I		
15	June 19	I	I?	I	I	2a	2a	2a	I?	I?	I?
	20	I	I	I	I	2a	2a	2a	I	I	I
	21	I	I	I	I	2a	2a	2a	I	I	I
	23	I	I	I	I	2a	2a	2a	I	I	I
	27	I	I	I	I	2a	2a	2a	I	I	I
	July 8	I	I	I	I	2a	2a	2a	I	I	I
16	June 19	I	I?	I	I	2a	2a	2a	I?	I?	I?
	20	I	I	I	I	2a	2a	2a	I	I	I
	21	I	I	I	I	2a	2a	2a	I	I	I
	23	I	I	I	I	2a	2a	2a	I	I	I
	27	I	I	I	I	2a	2a	2a	I	I	I
	July 8	I	I	I	I	2a	2b	2b	I	I	I
17	June 19		2a	2a	2a		2a	2a	2a	2a	2a
	20	I?	2a	2a	2a	2a?	2a	2a	2a	2a	2a
	21	I	2a	2a	2a	2a?	2a	2a	2a	2a	2a
	23	I	2a	2a	2a	2a?	2a	2a	2a	2a	2a
	27	I	2b	*	2a	3	3	*	-	-	-
	July 8	I	2b		2b	3	3				
18	June 19	2a	2a	2a	2a		2a		2a	2a	2a
	20	2a	2a	2a	2a	2a	2a	2a	2a	2a	2a
	21	2a	2a	2a	2a	2a	2a	3	2a	2a	2a
	23	2a	2a	2a	2a	2a	2a	3	2a	3	3
	27	2a	2b	*	2a	4	3	3	2b	*	3
	July 8	2b	2b		2b	4	3	3	2b		3
19	June 19	2a	2a?	2a	2a				2a?	2a	
	20	2a	2a?	2a	2a	2a	2a?	2a	2a?	2a	2a
	21	2a	2a?	2a	2a	2a	2a?	2a	2a?	2a	2a
	23	2a	2b	2a	2a	2a	2a?	3	2a?	3	3
	27	*	2b	*	2a	4	4	3	3	3	3
	July 8		2b		2b	4	4	3	3	3	3

\* Removed.

TABLE 1—Continued

PLANT INOCULATED		3a	3b	3c	3d	3e	3f	3g	3h	3i	3j
Leaf	Date										
20	June 19	3									
	20	3	2a?	2a?	2a?		2a		2a	2a	
	21	3	3	*	3	2a	2a		2a	2a	2a
	23	3	3		3	4	4		4	4	4
	27	3	3		3	4	4		4	4	4
	July 8	3	3		3	4	4		4	4	4
21	June 23		4	2a	3		4			4	
	27	4	4	4	3	3	4		4	4	4
	July 8	4	*	4	3	4	4		4	4	4
22	June 23		4	2a	2a		4?				
	27	4	4	4	4	4	4		4	4	4
	July 8	4	*	4	4	4	4		4	4	4
		5		5							
23	June 27		4		4	4			4	4	4
	July 8	5	4	4	*	4	4		4	4	4
24	June 27		4		4	4	4		4	4	4
	July 8	5	4								
			5	5	*	4	4		4	4	4
25	July 8	5		5	4						
26	8		5	5	4						
					5						
27	8	5	5	5	4						
					5						
28	8	6	6	5	5						
29	8	6	6	6	6						
30	8		6	6	6						
31	8			6	6						

\* Removed.

will show identical patterns, indicating a corresponding degree of reaction to the virus, and these mosaic patterns are correlated as they appear with the age of the leaves, their distance from the base of the plant, and from the inoculated leaf and the critical leaf.

The data given in TABLES 2 and 3 are from two other series of ten plants each. The plants were somewhat dwarfed, and were forced to early maturity under abnormal conditions of nutrition. The plants were only six inches tall, and growing in seven inch pots in a slightly pot-bound condition at the time of the experiment. In these two lots each plant was inoculated in

TABLE 2

*A record of the observations as to the time of the appearance of disease symptoms in ten plants, each inoculated in a different leaf, with the virus obtained from the same diseased leaf. The data are from the five plants of the ten which became diseased. The inoculations were made April 27. Plant 1a, which was inoculated in the basal leaf, 1b in the second leaf, 1c in the third leaf, 1d in the fourth leaf, and 1e in the fifth leaf did not become diseased. The controls 1m, 1n, and 1o remained healthy.*

PLANT	LEAF INOCU- LATED	MAY 16		MAY 17		MAY 19		MAY 20	
		DISEASED LEAVES	PATTERN	DISEASED LEAVES	PATTERN	DISEASED LEAVES	PATTERN	DISEASED LEAVES	PATTERN
1f	6					11	Type 1	11	Type 1
						14	" 2a	12	" 1
								14	" 2a
								15	" 2a
1g	7							17	" 2a
				13	Type 1	13	Type 1	13	Type 1
				14	" 2a	14	" 2a	14	" 2a
				16	" 2a	15-19	" 2a	15-19	" 2a
1h	8			17	" 2a?				
		17	Type 1	17	Type 1	14-17	Type 1	12-17	Type 1
		18	" 2a	18-20	Type 2a	18-20	" 2a	18-20	" 2a
		19	" 2a?						
1i	9	14	Type 2a	14-18	Type 2a	11-13	Type 1	11-13	Type 1
		16	" 2a			14-18	" 2a	14-18	" 2a
		17	" 2a						
1j	10	12-14	Type 1	11-14	Type 1				
		15	" 2a	15	" 2a				

TABLE 3

*A record of observations as to the early stages of disease in ten plants each inoculated in a different leaf, with the virus obtained from the same diseased leaf. Plants 2a, 2b, 2c, 2d did not become diseased. The controls 2k, 2l, and 2m remained healthy. The inoculations were made April 27.*

PLANT	LEAF INOCU- LATED	MAY 16		MAY 17		MAY 19		MAY 20	
		DISEASED LEAVES	PATTERN	DISEASED LEAVES	PATTERN	DISEASED LEAVES	PATTERN	DISEASED LEAVES	PATTERN
2e	5							13-15	Type 1
2f	6							16, 17	" 2a
						11, 12	Type 1	11, 12	Type 1
2g	7					14	" 2a	13, 14	" 2a
				12	Type 1	11-13	Type 1	11-13	Type 1
2h	8			15	" 2a	14-17	" 2a	14-17	" 2a
		16	Type 2a	15	Type 1	12-15	Type 1	12-15	Type 1
				16	" 2a?	16-18	" 2a	16-18	" 2a
				18	" 2a				
2i	9	10, 11	Type 1	10-12	Type 1	10-12	Type 1	10-12	Type 1
		13, 15	" 2a	13, 15	" 2a	13-17	" 2a	13-17	" 2a
2j	10	15	Type 2a	12-14	Type 1	11-14	Type 1	11-14	Type 1
				15-17	" 2a	15-17	" 2a	15-17	" 2a

a different leaf, that is, plant 1a in the basal leaf, plant 1b in the second leaf from the base of the plant, etc. In the second series similarly, plant 2a was inoculated in the basal leaf, plant 2b in the second leaf from the base, etc.

The disease appeared as clearly defined or barely apparent symptoms in one or several leaves below the critical leaf on the day it showed typical symptoms of type 2a (pale crinkled). The mosaic patterns soon became more marked and followed the normal course of development. In a number of cases during the early development of the disease, one or more leaves above the critical leaf showed no signs of the disease for several days. Such leaves often occurred vertically above one another along the same sector of the plant stem. In every case however, a few days later such leaves too showed symptoms belonging to pattern types 2a (pale crinkled) or 3 (narrow nervisequum).

TABLE 4

*Shades of color referred to in the leaf patterns described, with percentages of color of color in each, according to Ridgway's 'Color standards and nomenclature'*

NAME OF SHADE	PLATE	YELLOW	GREEN	COLOR	BLACK	COLOR	NEUTRAL GRAY
Dark dull yellow							
green	XXXII	11	89	12.5	87.5	42	58
Forest green	XVII	39	61	12.5	87.5	68	32
Light cress green	XXXI	39	61	55.0	45.0	42	58
Cress green	XXXI	39	61	29.5	70.5	42	58
Dark cress green	XXXI	39	61	12.5	87.5	42	58

The first two columns of the table represent the percentages of yellow and green (the component colors) found in the greens of the tobacco plant. The two central columns represent the percentages of pure color contained in each shade and the amount of black producing the differences in the luminosity of the shade, as determined by the color wheel measurements. In the third series, the percentage of color and of neutral gray contained in the shade are given.

#### *Glossary of terms used in connection with mosaic disease symptoms*

*Blotching*: a large irregular spotting, the spots either abnormally dark green or abnormally light green. [Century Dictionary.]

*Chlorosis*: a yellowing or paleness that affects the leaf blade, and is assumed to be diffused unless designated as mottling. [Schultz and Folsom.]

*Contortion*: a twisting of the leaf blade. [Schultz and Folsom.]

*Crinkled*: marked with short waves or wrinkled, less pronounced than wrinkling. [Century Dictionary.]

*Curling*: an abnormal bending of the leaf downward toward the main stem. Schultz and Folsom.]

*Dwarfing*: essentially a reduction in the size of parts rather than in their number, though both may occur together. [Schultz and Folsom.]

*Frenching*: abnormal sickly plants, the leaves of which may be free from mottling, stringy, very thick and leathery. [Allard.]

*Masking*: new leaves lacking all symptoms of the disease, of a uniform dark color, not mottled, or crinkled. [Allard.]

*Mottling*: a localized chlorosis consisting of spotting of the leaf blades by light green areas, which may or may not be in contact with the larger veins, and which may vary in shape and degree of paleness. The discolored spots may be punctate, elongate, circular, angular, irregular in form; varying in color from barely perceptible fading of the green color to an almost pure yellow. [Schultz and Folsom.]

*Necrosis*: the premature death of tissues, accompanied and manifested by their turning brown (e.g. spot necrosis, streak necrosis, etc.). [Schultz and Folsom.]

*Rolling*: an upward curving of the sides of each leaflet, with the midrib at the bottom of the trough thus formed. [Schultz and Folsom.]

*Ruffling*: an abnormal unevenness of the leaf blade surface caused by ridges that develop or become more pronounced with passage from the midrib to the lateral margins of the leaf, resulting in waviness of the margins. [Schultz and Folsom.]

*Savoying*: leaf surface with irregular rounded surface depressions and elevations.

*Wrinkling*: an abnormal unevenness of the leaf blade surface due to more or less ridge and furrow-like depressions and prominences not arranged in any uniform manner. [Schultz and Folsom.]

### III. A HISTOGENIC STUDY OF THE LEAVES OF HEALTHY AND DISEASED PLANTS

The earlier investigators were uncertain as to which regions of the mottled leaf, the dark green or pale green areas, were the diseased parts. Mayer (1886) thought it was the yellowed areas, Beijerinck (1889) the green. Iwanowski (1903) reported that the virus was more or less present throughout the leaf tissue, no matter whether dark or light green. He found that the juices of expressed yellow tissues produced infection in eight plants out of eight, but that the juices from the green areas gave infection in two out of the eight plants inoculated, and concluded that the disease is more localized in the light green areas. Iwanowski also was the first to show that a sharp histological differentiation exists between the yellow and green areas as shown in leaf sections, and that the transition between the two areas may occur within the extent of as few as two or three rows of cells. He showed that in the green areas the palisade and parenchyma are well developed, but that in the



thinner yellow areas there is no differentiation of palisade cells, and there are no intercellular spaces. He found that the cells in the yellowed areas are poor in chloroplasts, of which many are swollen and disintegrated; the cells here are a pale green color, their chloroplasts pale yellow, with not a single starch grain showing when iodine is added. He found that the cells of the yellowed areas contain crystalline plates which show cross striations upon the addition of Flemming's medium fixing solution. The nucleus in these areas is described as generally appearing normal and rounded, though somewhat large. Closely applied to the nuclei, he often found what appeared to be a parasitic amoeboid body which he suggested may be a second nucleus produced by amitotic nuclear division.

Dickson (1922) found that a striking difference in thickness, of the leaf blade exists between the light and dark areas. In the light areas the palisade parenchyma cells do not lengthen, or do so to a limited extent only, depending upon the severity of the infection. The cells of these areas are isodiametric, and there is no differentiation into palisade and mesophyll tissues. The intercellular spaces are much reduced. In the dark areas, the palisade cells form two layers instead of one, with the lower layer composed of somewhat shorter cells than the first. Iwanowski (1903) did not describe the presence of two palisade layers in his sections. Dickson regards this double palisade layer as hyperplasia, the two layers of palisade cells constituting a hyperplastic growth resulting from a slight or late stimulation of the meristematic cells. In the case of the original meristematic cells in the yellow areas where the infection is severe, and the palisade cells remain cuboidal in shape, hypoplasia is said to be present.

Rawlins and Johnson (1924) state that leaves in which the palisade layers and chloroplasts are well developed will never become badly mottled, and that part of the chlorotic appearance in mottled leaves seems to be due to the failure of the palisade cells in the chlorotic areas to elongate.

Rand (1923) from histological studies of the pecan rosette disease, found a distinct correlation existing between the external malformation of the leaves and the severe internal derangements of the tissues of those leaves. He found, in the most severely infected leaves, that there is no differentiation into palisade and

spongy tissue, and that the centers of the yellow spots consist of closely packed isodiametric cells without any intervening air spaces.

I find that Iwanowski's and Dickson's statements as to the histology of the dark green areas are not incompatible. Both conditions can be found, depending on the age of the leaf at the time when infection took place. My sections through mature healthy leaves of mature plants show that in tobacco as in many other plants, a second layer of less elongated palisade cells may normally occur. The term hyperplasia as implying the development of a second layer of palisade cells should not be used in connection with the dark green areas of diseased tobacco leaves. These areas consist of seven layers of cells such as are characteristic of healthy mature leaves.

The cells found in sections of the light green areas do constitute a hypoplastic condition due to severe infection, and represent the original embryonic condition of the leaf at the time when infection took place. Cytological study of very young leaves leads unquestionably to the conclusion that the entrance of the virus interferes with the histogenic development of the leaf. Embryonic cell layers of leaf primordia, which have been given an opportunity to develop before the entrance of the virus, show a normal palisade development. Furthermore, I find that the dark green areas, contrary to the opinion of Dickson, are in reality also hypoplastic, and show a cell form condition characteristic of a young stage in the development of a normal leaf. The cells in such infected leaves soon cease to differentiate, and the palisade cells have simply elongated, producing the double palisade layer whose cells never attain the size of the cells found in mature healthy leaves. In accord with Küster's (1904) view of hypoplasia, such an arrested development should be considered as representing a hypoplastic condition.

#### *Stages in the histogenic development of healthy tobacco leaves*

The following outline presents briefly the characteristics of anatomical structure found in certain stages of histogenic development in healthy leaves.

In the first stage, the leaf primordium consists of isodiametric meristematic cells, paralleliped in form in the dermatogen,

polygonal in the periblem. Cell division is confined to the apical meristem for a very short time, and then division figures may be found here and there through the extent of the primordium.

The second stage marks the beginning of intense intercalary division and the appearance of vacuoles in the cells, so that they lose their meristematic appearance, appearing multivacuolated and cuboidal in section. The nuclei are still centrally placed. The leaf primordium consists of six layers of cells, which all appear very much alike.

The third stage marks the first notable cell differentiation which will bring anatomical differences in the primordial histogenic layers which originally formed the primordium. A layer of palisade cells is formed in the first periblem layer below the upper surface of the leaf, by an increased number of radial divisions of the cells. The cells so formed are 25 by 10 microns. The continued radial divisions have cut up the original cuboidal cells into oblong segments. The plastids now are conspicuous and are distributed along the strands of cytoplasm which radiate from the nuclei. Active cell division in certain regions, distributed at equal intervals along the leaf in the second and third periblem layers, brings about the formation of groups of small polygonal cells, which are the rudiments of the veins of the leaf blade.

In the fourth stage, the palisade cells are further differentiated by elongation in a radial direction. Elongation in a radial direction in the second layer goes on to a less degree. The other two central layers enlarge in a plane parallel to the surface of the leaf. A seventh layer of cells is next formed by a series of cross divisions in the cells of the fourth layer. The cells are at this stage all univacuolate, and the plastids, which are now larger and more numerous, are found in the primordial utricle which bounds the walls.

In the fifth stage there is a well developed palisade layer, below which a layer of somewhat elongated cells forms a secondary and less differentiated palisade layer. The spongy parenchyma has completed its development, and large intercellular spaces have been formed between the elongated and somewhat lobed mesophyll cells, as the stretching of the blade during simple expansion of the cells in the layers has forced the in-

dividual cells somewhat apart. They still however remain in contact along the regions of the walls originally present in the early stages of histogenesis.

Stage 6 is only found in the later leaves to develop upon a mature plant. The leaf has attained complete maturity, with two well defined palisade layers, large well developed air spaces between the mesophyll cells, and the presence of numerous large plastids along the cell walls.

### *Cytological study of diseased leaves*

The time at which infection has taken place in a leaf of a diseased plant can be ascertained by a study of its anatomical structure. By sectioning diseased leaves of various sizes and various patterns described in a previous section, and comparing their anatomical structure with those of healthy leaves of corresponding sizes, a correlation between anatomical structure and disease pattern is found to exist. A correlation has been shown to exist between the leaf pattern and the stage of development of the leaf at the time of inoculation of the plant. In the same way a correlation can consequently be shown to exist between all three of these factors, age and size of leaf, anatomical structures of yellow and green areas, and the leaf pattern exhibited by the leaf.

I find that the presence of the virus in the early stages in the histogenic development of the leaf results in the more severe types of mosaic patterns. Sections through the diseased leaves of tobacco plants, at all stages of their development, show that histogenic development was arrested in every case by the entrance of the virus. The distribution of the virus in the tissue is indicated by the stage in histogenic development which the various cell regions show. The lighter green regions of the leaf patterns were invaded before the dark green or normal green areas, which have continued their histogenic development to a later stage. Sections through mature leaves, infected during the final stages of leaf development, show that the pattern is due to the effects of the disease upon the cell contents. In certain areas distributed through the leaf blade, such infections of leaves in later stages of histogenic development result in the faintly mottled appearance characteristic of pattern type 1 (dark, vaguely blotched) and type 2 (pale crinkled).

I find that the effect of the virus on the leaf tissues is much more diversified than is recognized by Iwanowski or Dickson.

1. Cytology of leaves showing type 1 pattern (dark, vaguely blotched)

In the case of leaves which are nearly full grown and above the point of inoculation, if they are young enough, the reaction of the cells will be visible in a barely perceptible mottling with yellowish green of the normally dark green cells. In a leaf infected when nearly mature, the light green areas may contain a double layer of palisade cells as well developed as that in the dark green areas. Such patterns as types 1 and 2a and 2b are the result of infections after the completion of the histogenic development of the leaves. In such leaves the only effects of the disease are upon cell contents. The plastids in sectioned material usually appear as in a normal leaf, though I have observed that in these regions of lighter green there is a distinctly different staining reaction visible, when the sections are stained with the Flemming's triple stain. The mottling seen in such leaves is probably due to a slight loss of green in the chloroplasts. I have further observed a difference in the staining reaction, which varies along the section, the darker green areas taking a more intense blue stain, while the lighter areas appear more red or orange. The cells of all the regions show the presence of striated bodies and x-bodies in these leaves.

Plate 23, figures 1a, 1b, and 1c, are drawings from sections of a large leaf 12 X 6 inches, which showed pattern type 1 (dark, vaguely blotched), and from which the pieces of material for fixation were cut across the vein islets so as to include the lighter and darker regions. The histogenic development was found to be that of stage 5, in which a single well elongated palisade layer is present, the layer beneath this having lost its palisade-like characters of an earlier stage (stage 4) through a failure to continue to elongate, and by a separating of the individual cells, leaving large intercellular spaces between them. Seven layers of cells are found throughout the leaf, and large intercellular spaces occur between the cells of the mesophyll layers. In spite of the large size of the leaf, this is the anatomical structure to be expected, as it is not until the plant is completely mature that the leaves which then are developing upon the

stem will show the stage of histogenic development (stage 6) in which two well recognized palisade layers are present.

The cell structure in such a leaf is quite normal, though the x-bodies and striated bodies are present in the cytoplasm. Figure 1a is a drawing of a palisade cell from a cross section of this leaf. The nucleus of the cell is distorted by the pressure of an x-body against it. A striated body lies against the side wall, while another striated body, cut across the lines of striations, appears as a somewhat granular mass above it. The plastids are plump, and lie along the cell walls in the primordial utricle, and show no starch grains, although the material was fixed at five o'clock in the afternoon.

Figure 1b is a cell from the third layer of cells in the same leaf. The disorganized striated material has dissolved to such an extent, perhaps due to overheating of the material during the processes of fixation, or to some chemical state or condition of the crystal itself at the time of fixation, that the individual fibers or striae nearly fill the cell lumen. The plastids are present, lying against the lower wall of the cell as rounded faintly stained bodies. Figure 1c is that of an epidermal cell showing the presence of a large nucleus and a vacuolate x-body lying below it.

## 2. Cytology of leaves showing pattern types 2a (pale crinkled) and 2b (pale, vaguely blotched)

Those leaves on the plant which are the youngest at the time of inoculation, and therefore capable of extensive growth, show disease pattern type 2a (pale crinkled). In these leaves histogenic development has also been completed, as in the older leaves showing type 1 (dark, vaguely blotched), but the cells are not as large nor the palisade cells anywhere nearly as much elongated. These younger cells show the effects of the disease in a more pronounced manner. The plastids are fewer and they are much smaller in size than those found in type 1 (dark, vaguely blotched). This explains the lighter green color of the leaf, since the plastids are fewer in number and contain less starch, and the striated crystals and x-bodies are more numerous. In prepared material, the only visible sign of the mottling found in the living leaf is present in the staining reactions along the section of the blade. In the paler green areas which mark

the center of the vein islets, when the Flemming's triple stain is used, the sections show a greater tendency to stain orange and red, while in the darker green areas there is more of the blue of the gentian violet evident.

Figure 2, plate 23, is a drawing of a palisade cell from a leaf  $8.5 \times 3.75$  inches in size, which showed the intensely crinkled light veined pattern and light green color of a leaf of type 2a pattern (pale crinkled). Sections of this leaf showed the presence of only six layers of cells. The air spaces between the mesophyll cells are still very small. The palisade cells are well developed, but much shorter than those in a leaf showing type 1 pattern (dark, vaguely blotched) described before. The development of this leaf seems to be at stage 4 of histogenesis. This palisade cell showed two striated crystals, upon which the nucleus lies. The plastids contain starch grains, and these are in the form of lens shaped, dark blue stained grains in the colorless stroma of the plastid. The thin cytoplasmic sheath that bounds the plastids is visible around each one. As has been explained, the difference between the slightly darker and lighter green areas of this leaf is only apparent from the darker blue staining reaction of the cells in the darker green area. This may be explained as the result of the presence of more chlorophyll in the plastids of this region, resulting in their better functioning and the presence of more starch.

When such a leaf upon further expansion loses its intensely crinkled effect, the result is due simply to the growth of the individual cells, which have increased in width instead of radially to form a more elongated palisade layer characteristic of more mature leaves. The anatomical structure remains the same, save that numerous intercellular spaces appear between the cells of the mesophyll, and even between the cells of the palisade layer. The plastids are slightly smaller, and the nuclei more swollen. A great deal more of the striated material is found, and the x-bodies are very numerous and quite large.

Sections of a leaf  $10 \times 4.5$  inches, showing type 2b pattern (pale, vaguely blotched) that is, a 'light cress green' leaf in which the regions of lighter and darker green are scarcely differentiated, and one in which the earlier intense crinkling is considerably lost, show seven layers of cells, with well developed air spaces between all the cells of the inner five layers. The

palisade cells are much wider, but only slightly longer than in the earlier stage, type 2a (pale crinkled). Large masses of yellow staining material, the disorganized striated bodies, are found throughout the sections. The nuclei are large and swollen, and irregular in outline. Figure 3a, plate 23 is of a palisade cell showing the nucleus somewhat buried in a similar mass of material from the striated body, and a very large vacuolated x-body extending across the cell vacuole. The plastids appear vacuolated, but the apparent vacuoles are only lens-shaped starch grains into which the stain has not penetrated, owing either to the changed nature of the starch so that it does not take the gentian stain, or overheating during some stage of imbedding or mounting. Figure 3b is a cell of the third layer, showing a tendency of even this third layer of cells to elongate in the direction of the surface of the leaf blade, rather than radially, to produce another palisade layer. Figure 3c is a drawing of an epidermal cell showing the presence of an amoeboid shaped nucleus, an x-body with a large vacuole, and a broad striated body.

The presence of seven layers of cells in sections of a leaf showing type 2b pattern simply means that there had been seven layers of cells present when the leaf first became diseased. Six or seven layers of cells may be found in leaves showing type 2a (pale crinkled) or type 2b (pale, vaguely blotched) according to the age of the leaf when infection took place, and whether or not the formation of the seventh layer of cells during the fourth stage of histogenesis has taken place.

As has been explained before in a previous section on symptomatology, dark areas often become visible upon the further expansion of a leaf showing type 2a pattern (pale crinkled). This means that a few isolated cells have retained their healthy condition and present the dark green color of a normal leaf. In such areas the cells show plastids that are larger, with a richer starch content, and an otherwise normal cell content.

When sections are made of healthy leaves of size corresponding to these described as showing pattern types 2a and 2b, they show a histogenic development similar to that described for those leaves. In sections of a leaf that was  $7.5 \times 4.5$  inches in size, there are six layers of cells. The sections show a single palisade layer, and small air spaces developed between the



mesophyll cells. The anatomical structure corresponds very closely to the description given for a leaf showing type 2a pattern. Sections through a healthy leaf  $9 \times 5$  inches show a later histogenic development. There are present seven layers of cells, but still a single palisade layer is found, since this leaf was produced upon a vigorously growing healthy plant only four inches high, before the mature type of anatomical structure is produced in the later leaves on the plant. The cells are larger, and in the palisade layer much longer than those described for a smaller leaf. Figure 4 is a drawing of a palisade cell showing the elongated oblong outline characteristic of the cells of this layer in healthy leaves. The plastids, because of over-staining with the orange G of the triple stain, appear almost entirely orange, save for small blue or reddish-blue staining starch grains still retaining the gentian or safranin stains.

Sections of healthy leaves upon a plant that is young and vigorously growing will show a histological development and anatomical structure no different from that of the leaf just described. When figure 3a (palisade cell from a leaf showing type 2a pattern) is compared with figure 4 (palisade cell from a healthy leaf of corresponding size) the similarity in form and cell structure is clearly evident.

### 3. Cytology of leaves showing pattern type 3 (narrow nervisequum)

The youngest leaves on the plant at the time of the inoculation, which unfold during the initial appearance of the disease, as has been explained before, often show a pattern that is mistaken at first for type 2a (pale crinkled). In a few days however, the crinkled effect is entirely lost, and the leaf becomes perfectly flattened out into a smooth blade and shows the presence of numerous dark green narrow bands along the small veins, so that it becomes clearly evident that the leaf belongs to type 3 pattern (narrow nervisequum). That this pattern is a close transition between that of type 2 and type 4 is evident from cytological studies. For example, sections through a leaf  $7.5 \times 3$  inches of type 3 pattern (narrow nervisequum) may show an anatomical structure very similar indeed to that described for types 2a (pale crinkled) and 2b (pale, vaguely blotched). There are seven layers of cells, including a single

palisade layer, and small air spaces between the mesophyll cells. The dark green areas show the presence of healthy cells with normal plastids, while the light green areas present the same appearance as such cells shown in figures 3a, 3b, and 3c.

On the other hand, sections through leaves of this same type may show the distinct differences in anatomical structure that are so characteristic of the light and dark green areas of leaves showing type 4 pattern (malformed, broad nervisequum).

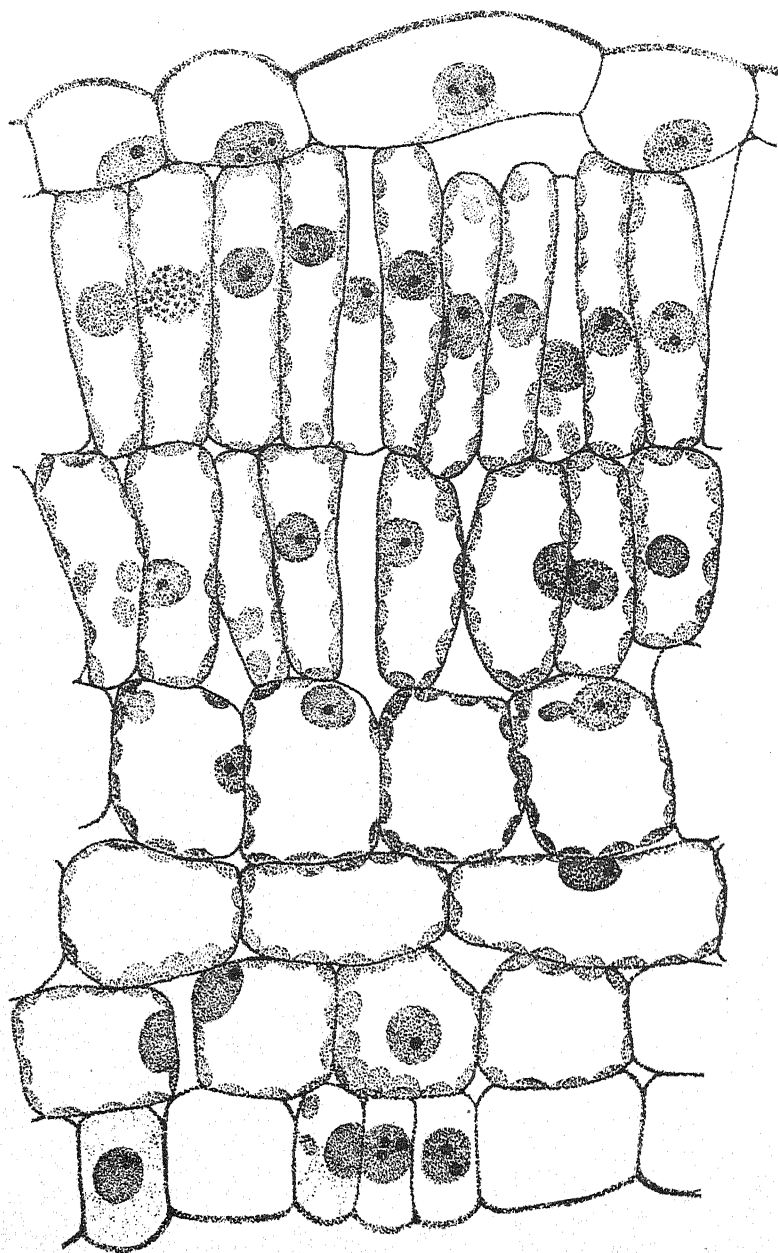
Sections through a leaf  $5 \times 2$  inches showing type 3 pattern (narrow nervisequum) showed very pronounced differences between the dark and light green areas. This leaf evidently had been infected at a much younger stage of histogenic development than had the larger leaf. This difference between the anatomical structure found in the dark and light green areas shows that the leaf was infected when it had attained very little histogenic development. Since these leaves are not distorted, deformed, or contorted in any way, as are the leaves of type 4 (malformed, broad nervisequum) it is evident that they were not infected in the primordial stage when still very close to the growing point, but when they had already developed into small leaves, about .5 to 1 inch in length at least. Practically the entire leaf is diseased, as sections show, with only the dark green narrow bands along the veins left to continue their histogenic development up through stage 4 of histogenesis.

A section through a narrow dark green area and extending out into the light green area shows a remarkable contrast in the thickness of the two areas, and in their anatomical structures. In the dark green area, the structure attained is that of histogenic stage 3 or 4 in a healthy leaf, that is, there are six or seven layers of cells, and the first palisade layer is distinctly developed, while the third layer of cells has elongated somewhat, and appears as a feebly developed second palisade layer. The cells of the first layer are however about twice as long as those of the second layer, though the latter cells, because of their radial elongation and closeness to one another, suggest a typical palisade development. The air spaces, on the other hand, are feebly developed between the mesophyll cells, which seem to be more regularly cuboidal than is characteristic of the mesophyll cells of healthy leaves. The nuclei in this dark green region are much smaller in diameter, 9.44 microns (average of fifty micrometer measure-

ments) than are those of the light green areas, 10.24 microns. The plastids of the dark green areas are much larger, 7.16 microns, than those of the light green areas, which only measure 7.04 microns in diameter. All the cells of this region show a cell content normal for the histogenic development attained, that is a univacuolate cell, with plastids confined to the primordial utricle. The material was not fixed at a suitable time to demonstrate the presence of starch grains, so that the plastids are present as evenly staining orange bodies, quite plump and numerous.

The light green areas, which in this pattern (narrow nervisequum) occupy the greater part of the leaf blade, are composed of six layers of cells as seen from sections, with no distinguishable anatomical differentiation into palisade and mesophyll layers. The cells of these regions are more or less irregularly cuboidal, with small irregularly occurring intercellular spaces. The transition between the two regions is very abrupt, there often being found only two or three palisade cells, which by their shorter length and increasing width indicate the transition to the more or less cuboid form of the palisade cells of the yellow areas. The cell contents of the cells of the light green areas are also strikingly different. The four inner layers, which normally show numerous plastids, show relatively few plastids along the walls, and these when present are distinctly smaller than those of the dark green areas. The plastids in the dark green area show a diameter of 7.16 microns (average measurement of 50), while those in the light green areas measure only 7.04 microns. The nuclei in the light green areas are much larger than those in the dark green areas and appear decidedly hypertrophied in many of the cells. The average diameter of the nuclei in the dark green areas is 9.44 microns, while the average diameter of those in the light green areas is 10.24 microns. Nearly all the cells show the presence of the disorganized striated bodies and x-bodies, usually lying in close contact with the nucleus. Text-figures 1 and 2 represent drawings of sections through the dark and light green areas of a leaf of type 3 pattern (narrow nervisequum), showing such characteristic differences as I have already described.

Figure 5a is a palisade cell from a dark green area, showing the plump plastids arranged in close proximity around the cell



TEXT-FIG. I.

walls, and the cell nucleus in this case suspended in the cell vacuole. Figure 5b is a cell from the second palisade layer, which differs only in length from the first palisade cell. Figure 5c shows a palisade cell from the first layer, which is one of a group of three cells which alone marked the transition from the dark green to the light green regions. This cell is nearly twice as wide as the normal palisade cell, and shows cell contents characteristic of a much earlier stage in histogenesis, that is the presence of very few plastids, distributed along the cytoplasmic threads which suspend the nucleus in the center of the cell. Figure 5d shows a typical cell form for a cell from the palisade layer of the light green area: the plastids are few and scattered; the nucleus is larger than normal and lies partially surrounded by a striated body, with an x-body lying beneath it; the x-body shows two vacuoles, and in one of these there is a distinctly red stained minute granular mass of material, with the slenderest of threads radiating from it; the vacuole is distinctly contrasted with the dense material of the body around it. The cells of the second layer are not strikingly different from those of the first layer.

4. Cytology of leaves showing type 4 pattern (malformed, broad nervisequum): a comparative study of sections from healthy and diseased leaves of various sizes and ages, in which infection occurred while the leaves were in the primordium stage

1. *Introduction.* In an inoculated plant, the inoculated leaf, if not fully grown at the time of the inoculation, and several leaves above it show a checking of growth much sooner than do corresponding leaves of healthy plants. The youngest leaves of inoculated plants sometimes appear for a time to grow just as rapidly and continuously as those of healthy plants, but they never attain the size of corresponding leaves of healthy plants. The younger leaves of diseased plants are regularly narrower than those of healthy plants.

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TEXT-FIG. 1. The dark green region of a leaf showing type 3 pattern (narrow nervisequum). It has reached an anatomical structure or development characteristic of stage 4 in histogenesis: seven layers of cells are found, such as are also characteristic of healthy leaves at this stage of development; there are two young developing palisade layers; the intercellular spaces are only feebly developed.  $\times 845$ .

If a leaf is infected during the primordial stage at the growing point, it may be so checked in its histogenic development that only a very narrow leaf blade will develop, or perhaps none at all, so that the leaf appears as a midrib only. This phase of frenching is characterized as shoestrings. In the early stages of the development of the tobacco leaf primordium, the sections of the primordia show a greatly enlarged midrib, with comparatively little blade developed on either side of it. The large size of the midrib of the leaf, with the further development of the blade halted by the presence of the virus, results in the blade appearing only as a slightly ragged edge along each side of the midrib, or scarcely at all.

Wingard (1924) describing 'frenching or shoe strings' of tobacco plants, asserts that, although frequently confused with mosaic, shoe strings is now known to be an entirely distinct trouble, and that the two diseases frequently occur on the same plant, and that this has led to the confusion. Narrowing of the leaves of tobacco plants may of course be caused by unfavorable conditions of the soil, defective drainage, deficiency of plant food, or lack of cultivation, nevertheless it is agreed by Allard (1914) Clinton (1915) and the earlier investigators that frenching is also a distinct symptom of the mosaic disease.

Frenched leaves may often be reduced to midrib alone. A study of the development of leaf primordia shows how this occurs. The first elongation of a leaf is due to repeated intercalary divisions in radial planes along all the layers of the blade. This is followed by a stretching of the blade, brought about by the elongation of the cells themselves, especially along the midrib of the blade. When the first elongation phase has ceased, the meristematic growth of the leaf margin may still continue for some time, before a simple stretching out in width of the cells occurs in all the layers. The earliest growth of a leaf appears to be mainly growth in length. The ratio of growth in length to growth in width changes as the leaf grows older. The virus entering a leaf at any of these stages in the development of the leaf will prevent the development in width of the blade, which belongs to the later stages of growth, and so produce a leaf with a very much reduced blade.

Frenched leaves are of an even 'dark dull yellow green' color in my cultures, and show no or very little mottling. When,

however, very young plants are inoculated, the first leaves appearing after the initial stages of the disease are decidedly frenched, but of a 'light cross green' color, with numerous very deep green bullate regions along their veins.

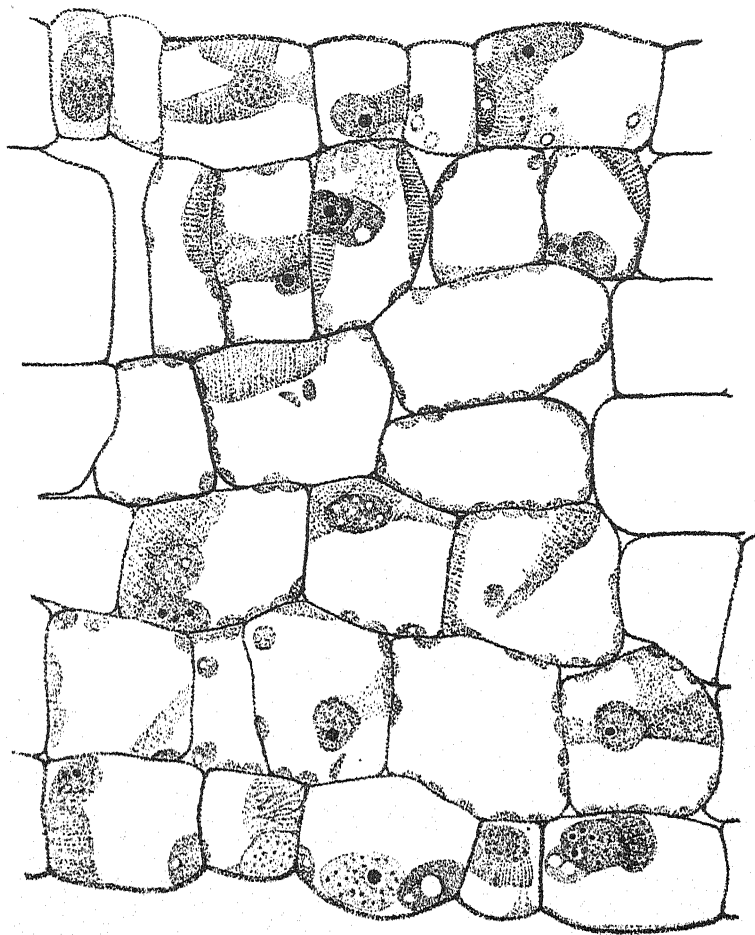


FIG. 2. The light green region of the leaf shown in TEXT-FIG. 1. Individual cells have developed histogenetically as far as the cell content only is concerned, and show a univacuolate cell, with small plastids imbedded in the primordial utricle. In form, however, this region has retained the embryonic cell structure of a leaf primordium, in which all the cells are somewhat cuboidal in shape, and in which there are only six layers of cells, and few intercellular spaces.  $\times 845$ .

That some portions of the leaf blade actually escape infection is evident from the broad or narrow bands of dark green which lie along the main secondary veins of leaves of type 4 pattern (malformed, coarse nervisequum). Such regions will often show a histogenic development far beyond that of the corresponding tissues of a healthy leaf of the same size as the diseased leaf. However the histogenic development in the adjacent light green areas has been stopped, and the cells remain more or less cuboidal in form, with slightly rounded corners that allow small intercellular spaces to appear. The cells show few plastids, numerous disorganized striated bodies, very numerous x-bodies, and large swollen nuclei. There are never more than six layers of cells present.

It is often apparent in leaves of this type that in one or more regions of the blade an area of rather extremely veined appearance is found. The veins tend to fuse laterally and form a very irregular network. The whole region presents a white veined or ridged appearance, while vein islets are reduced to a minimum in area. A section through such a region shows the presence of very numerous veins of all sizes and diameters, very close to one another with the tissue between the veins reduced to only a very few cells, which are very small, cuboidal, and sometimes very much diseased, or dead in appearance. Plate 21, figure 3, shows such white elongated veined areas within the dark green bands along the veins.

The veins are laid down very early in the development of the leaf, so that the entrance of the virus can hardly intercept their development. This early formation of the vein tissue is shown very clearly by Herrig in his figures of early primordium stages of leaves of *Hippuris* and other plants. When the disease becomes localized in such a region, the tissues between the veins, as seen from the form and size of the cells in the vein islets of this region, cease to undergo development, and remain practically identical in all respects with the cells of the primary tissues of the primordium.

2. *Histology of an infected primordium: first stage of histogenesis.* In sections from young leaf primordia into which the virus has penetrated very early, cell divisions seem to be quite normal, though the cells are more vacuolated than is the case in a healthy primordium of corresponding size. As is well



known and repeatedly shown in illustrations, the cells of the primordial layers contain large rounded nuclei, and are filled with fine granular cytoplasm, and never show the presence of vacuoles or cell inclusions of any kind. Figure 6 is a dermatogen cell from a primordium about 833 microns in length, and shows the presence of vacuoles even at this early stage. A striated body lies along the outer wall of the cell, and a small x-body lies between the nucleus and the lateral wall of the cell.

3. *Histology of a young leaf in the second stage of histogenesis.* When at this stage six layers of cells all alike in size and form are found, the cell content of cells in healthy and diseased leaves, and the cell form are very much alike. As shown in figures 7a and 7b, the cells are both somewhat cuboidal in form, and show radiating films and strands of cytoplasm cutting the cell up into many vacuoles. Figure 7a is a palisade cell from a diseased primordium, and shows a somewhat elongated and vacuolated x-body present in the cytoplasm. A vacuole of the x-body contains a minute red stained body with radiating threads, and the four small univacuolate bodies which lie about in the cytoplasm also show a similar structure within their vacuoles. Figure 7b shows a palisade cell from a healthy primordium in which the cytoplasmic material cuts across the cell in all directions dividing the cell space into several vacuoles. No plastids are present in this stage.

4. *Histology of a diseased and healthy leaf in the third stage of histogenesis.* Sections through a healthy leaf  $\frac{5}{8}$  by 3 inches in size show the third stage of histogenic development. The formation of the upper palisade layer is taking place at this stage, by means of radial divisions in the cells of the first periblem layer beneath the upper epidermis. The cells of the other layers are more or less cuboidal and still multivacuolate. The nuclei are smaller in comparison to the size of the cells than are the nuclei of meristematic cells. The plastids appear as rounded dense cytoplasmic knots along the cytoplasmic strands. These are still few in number, and occur in the epidermal cells, as well as in the cells of the periblem layers. Figures 8a, 8b, and 8c illustrate cells from such a healthy leaf section. Figure 8a shows a palisade cell elongated only slightly, but appearing elongated radially because of the radial division of the periblem cuboidal mother cell. Figure 8b represents a cell from the

second periblem layer which is still cuboidal. Figure 8c shows an epidermal cell from the upper dermatogen layer.

In sections of a small diseased leaf of the same size as above, but showing pattern type 4, with the broad dark green bands along the veins scarcely developed as yet, and only showing as very narrow scarcely darker green areas along the larger veins as shown in the photograph on plate 21, figure 2, the influence of the virus at this early stage in the histogenic development of the leaf is quite apparent. Although the darker green bands of the leaf are, as far as their cell content is concerned, still at the third stage of histogenesis as described in the proceeding paragraph, a greater elongation has taken place in the cells of the second and third layers from the upper surface, so that the cell form is characteristic of the fourth stage in histogenesis, where a first palisade layer is distinctly apparent, and a second less so in the third layer from the upper surface.

Figure 8d is a drawing of a palisade cell of the second layer of cells, from a section through a dark green area of such a leaf as shown in figure 2, plate 22. The plastids are strung along the cytoplasmic threads. Figure 8e shows a cell from the layer below, in which the marked elongation of the cells of this region is quite evident, when compared with a corresponding cell from a healthy leaf shown in figure 8b.

Figure 8f represents a cell from the palisade layer of the light green area, corresponding to a true palisade cell such as figured in 8d for the dark green area of the leaf, and in 8a for a healthy leaf. The cell has retained its embryonic cuboidal shape, owing to the failure to elongate radially and the occurrence of fewer radial divisions. The few plastids present are found in the primordial utricle. The nucleus is large and lightly stained, and shows the presence of fine chromatin threads and two small nucleoles. An x-body lies below it and below the large striated body. The striated body was cracked, probably in cutting, and shows this by a break in the striations. Figure 8g is a cell from the second periblem layer, which has retained more nearly the size of a corresponding cell of a healthy leaf shown in figure 8b. It offers an extreme contrast to the greatly elongated corresponding cell of the dark green area in figure 8e. Here the striated body has been cut transversely, across the lines of striation, so that a mass of small dark staining rod-like bodies

spread across the cell. The cytoplasm with imbedded nucleus is massed along the lower cell wall. A few plastids are found in the primordial utricle. No x-body is present in the cell.

Figure 8h is of an epidermal cell of the dark green area of the diseased leaf, and shows a more mature cell content than that of the healthy cell shown in figure 8c. The cell appears univacuolate, and the cytoplasm is not present in delicate radiating strands, such as are evident in the healthy epidermal cell. Figure 8i is an epidermal cell from the light green area, and shows a similar type of cell to that in the dark green area, but contains a small x-body and a striated body.

5. *Histology of a diseased and healthy leaf taken from the flower stalk, in the third stage of histogenesis.* Healthy leaves and diseased leaves showing pattern type 4 (malformed, broad nervisequum) taken from the flower stems again show great contrasts in cell form and cell content. The leaves examined were  $1 \times 3.75$  inches in size. The healthy leaf shows an early stage of histogenic development described as 3. The palisade cells are only slightly elongated, and are being formed by radial divisions in the upper second layer of cells. The plastids appear as described in the previous healthy leaf and figured in figures 8a, 8b, and 8c. The cells of the layer below the palisade layer appear as that shown in figure 8b. There are only six layers of cells present and the intercellular spaces are very small.

The palisade cells from the dark green area of the diseased leaf, although not much longer than the cells in healthy leaves, are decidedly a more mature type of cell, as shown in figure 9b. This is evident in the univacuolate condition, and the arrangement of the plastids, not along the cytoplasmic strands, as in figure 9a, but along the cell walls in the primordial utricle. Figure 9c shows a cell of the light green area that corresponds to the palisade cell of the dark green area figured in 9b, and the palisade cell from the healthy leaf figured in 9a: the cell is univacuolate, and the plastids are arranged in the primordial utricle; this cell contains a large x-body next to the nucleus. Evidently the only effect of the disease is on the cell form, that is, the cell has failed to elongate normally. Figure 9d shows an epidermal cell from the same light green region, in which the nucleus lies above a striated body which fills the lower half of the cell; a single plastid with small starch grains lies

above the striated body; in the cell vacuole at the top of the cell is a large x-body.

Seven layers of cells are found in the dark green areas of this diseased leaf, and the cell content development is at a stage of histogenesis far beyond that of a healthy leaf of the same size. There is a single central vacuole in each cell, and the plastids lie in the primordial utricle. Although there is yet no differentiation of a second palisade layer of cells, the cell structure of the third layer is as advanced as that of the cells in the palisade layer. The air spaces found between the mesophyll cells are much larger than those found between the cells of a healthy leaf at this stage.

In the yellow green areas, histogenesis seems to have stopped entirely and the anatomical appearance is distinctly embryonic. Six layers of cells are found, and the cells of all the layers are nearly cuboidal in shape, closely packed together, with only a very irregular appearance of air spaces. There is no differentiation into palisade and mesophyll tissue.

There is a distinct difference between the sizes of the nuclei in the cells of the healthy leaf and those of the dark and light green areas of the diseased leaves described here. The plastids also differ considerably. For example, the nuclei of the cells of the healthy leaf are smaller than those found in the light green area of the diseased leaf, but the nuclei of the cells of the dark green areas of the diseased leaf are even smaller than those of the healthy cells of healthy leaves. The average of 50 eye piece micrometer measurements of nuclear diameter showed that the nuclei of the cells in the healthy leaf  $1 \times 3.75$  inches were 10 microns in diameter, while the nuclei of the dark green regions of the diseased leaf averaged only 9.04 microns. However the nuclei in the light green areas showed an average diameter of 12.08 microns, an increase of 30.4 per cent over those in the dark green areas, and 20.8 per cent over those in the healthy leaves.

The plastids of the light green areas of diseased leaf cells may be only slightly smaller or very much so, according to the degree of virulence of the infection, than those found in healthy leaves. However the plastids of the dark green areas are decidedly larger than those of the light green areas, and also larger than those of the healthy leaf. The average diameter

of the plastids in the cells of the healthy leaf was 5.35 microns, while those in the dark green areas measured 7.74 microns. However, the plastids of the light green areas measured 5.32 microns in average diameter, which is only slightly smaller than those in the healthy leaf. The older the diseased leaf, the more apparent will be the reduction in size of the plastids of the light green areas when compared with those of the healthy leaf. The leaves from which the above measurements were taken were comparatively small and young, and the difference is not so marked. When measurements are given later for the plastids of older leaves in connection with the histogenesis of these leaves, the contrast will be found to be more marked.

6. *Histology of a diseased and healthy leaf in the fourth stage of histogenesis.* Sections of a healthy leaf  $2 \times 5$  inches and those of a diseased leaf of the same size showing pattern type 4 (malformed, broad nervisequum) reveal marked differences between the anatomical structure of the light green areas of the diseased leaf and that of the dark green areas. However the contrast between anatomical structure of the dark green areas of the diseased leaf and the healthy leaf is no longer as great as it was in smaller leaves.

Sections through a healthy leaf of this size show an anatomical structure characteristic of stage 4 in histogenesis. There are seven layers of cells in the section. The cells of the first palisade layer are 108 microns in length; the great elongation in this case is due to continual radial elongation. This is apparently a typical palisade structure of long, narrow, closely packed cells, with only the slightest of air spaces where adjacent cells meet. A slight elongation has also taken place in the third layer of cells, giving it the appearance of a very much less developed palisade layer. The cells of the mesophyll, on the other hand, have elongated in a direction parallel to the surface of the leaf, but do not show as yet any very large intercellular air spaces. The cell contents have become differentiated, as is characteristic of this stage in histogenesis. The cells are univacuolate, with the nucleus lying in the primordial utricle, or hung in the cell lumen by a few delicate cytoplasmic threads. The plastids as usual lie in the primordial utricle.

Figure 10a illustrates a palisade cell from the second layer of the leaf section, and figure 10b a cell from the third layer of

the section. This last figure shows the relatively wider and less elongated character of the cells of this layer when compared with those of the true palisade layer. Figure 10c is that of an epidermal cell showing a more developed stage of cell content than that figured in 8g. There has been no increase in the number of plastids in this epidermal cell of an older leaf, and the cells are univacuolate.

When the histogenic development attained by the dark green areas in the diseased leaf is compared with that of a healthy leaf, we find no difference. The precocious tendencies noted in connection with the younger leaves previously described is no longer evident. The healthy leaf, on increasing to this larger size, has had opportunity for continued histogenic differentiation. The smaller diseased leaves, when compared with smaller healthy leaves, had appeared to be further advanced histogenically, because in reality they were older leaves than the healthy leaves, even though of the same size. If the sections of these leaves had been made when they were smaller and younger, no doubt it would have been seen that the dark green area of the diseased leaf had attained a more advanced histogenic development. Figure 10d shows a palisade cell from the dark green area, and figure 10e an elongated cell from the third layer of cells, which, because of their elongated character, present, as in the healthy leaf, the appearance of a second layer of less developed palisade cells. The cell content of the two is the same. Both of these cells are slightly shorter than those of the corresponding cells of the healthy leaf.

Figure 10f shows a transition cell from the first palisade layer between the dark green and light green areas. This cell is slightly wider and shorter than the palisade cell in figure 10d, and although its plastids are still somewhat normally arranged, it shows the presence of a disorganized striated body and an x-body pressed against the nucleus. Figure 10g shows the corresponding palisade cell of a pale green area. It has not only retained its embryonic cuboidal form, but still shows the central nucleus and multivacuolate condition, and a few small plastids. A small x-body lies near the nucleus. Figure 10h shows a secondary palisade layer cell of the light green area. It is small and cuboidal in form, and its cytoplasm, nucleus, and an x-body are all massed in the lower part of the cell.

Again the same relationship can be found as to the relative size of the nucleus and plastids in the healthy leaf and the dark green and light green regions of the diseased leaf. The nuclei of the cells of the healthy leaf average 10.44 microns in diameter (50 nuclei measured). The nuclei of the dark green region of the diseased leaf are smaller, showing an average diameter of 10.18 microns, while the nuclei of the light green areas are slightly larger than the nuclei of a healthy leaf, showing an average diameter of 10.56 microns. The average diameter of the plastids of the cells in the healthy leaf is 7.5 microns. The plastids in the light green areas of the diseased leaf average only 5.7 microns, showing a much reduced and shrunken condition. The plastids in the dark green regions are larger, 6.48 microns, but not quite as large as those of the correspondingly healthy leaf.

The relative sizes of the nuclei and plastids of healthy and diseased areas of diseased leaves showing pattern type 4 (malformed, broad nervisequum) and those of healthy leaves of the same size, support the view that the dark green areas represent, not regions of hyperplasia, but, on the contrary, regions of arrested development. The nuclei of the dark green areas are always smaller than those of the healthy cells of healthy leaves. The plastid size indicates distinctly the inhibitive action of the disease. For, although I found that in the dark green areas of a diseased leaf  $1 \times 3.75$  inches the plastids are larger than those of a healthy leaf of corresponding size, this is due to the fact that the diseased leaf is precocious in histogenic development as compared with the healthy leaf, and is really much older. The plastids in a healthy leaf continue to increase in size until, in a leaf  $5 \times 2$  inches, they have become larger than the plastids in the dark green region of a diseased leaf of this size.

7. *Histology of a diseased and healthy leaf showing stage 6 in histogenic development.* In the later development of leaves upon a plant approaching maturity, instead of passing from stage 4—where two layers of palisade cells seem to be present—into stage 5—where only the first palisade layer continues to elongate radially and intensify the palisade characters, and in which the cells of the third layer cease to elongate, and begin to broaden and show the presence of large intercellular spaces,

so that only one distinct palisade layer is evident—the histogenic development passes from stage 4 directly to stage 6, where both palisade layers continue to develop, producing a double palisade layer.

In sections of a healthy leaf  $8 \times 4$  inches there appear two well developed palisade layers. The palisade cells of the first layer are about 66 microns in length, while those of the second layer are 33 microns in length. The intercellular spaces are well developed between the mesophyll cells, and to a slight degree between the palisade cells of the second layer. Figure 11d is a drawing of a cell of the first palisade layer, and figure 11e shows a cell of the second palisade layer. The cells show clearly the characteristic cell development in a leaf that has attained the histogenic development of stage 6, that is, a mature leaf borne upon a mature plant. Each plastid contains large starch grains, and the delicate cytoplasmic sheath in which the plastids are enveloped is visible in the section of the plastids only in the form of a thin semi-circular layer around each plastid. There is a single large central vacuole filling the entire cell, and the nucleus lies in the primordial utricle.

Figures 11a, 11b, and 11c show corresponding cells from a diseased leaf showing type 4 pattern. Figure 11a shows a palisade cell from the first layer, which corresponds to that of a healthy leaf as shown in figure 11d. The cell is about as long, and its cell content very similar. The cell shown in figure 11b, is a palisade cell from the second layer of palisade cells and resembles that of the corresponding healthy cell in figure 11e. Figure 11c shows the greatest contrast between the structure of the yellow green and dark green areas. This cell, although short and somewhat cuboidal in form, corresponds to a palisade cell of the first layer shown in figure 11a from the dark green area of the same leaf.

Sections through a leaf  $6.5 \times 3.25$  inches of the same pattern from a very old plant, show again a histogenic development very much like that of a mature leaf upon an old mature healthy plant. In the dark green areas of the diseased leaf, radial elongations had gone on to such an extent that one might say there were three palisade layers, as shown in figures 12a, 12b, and 12c. The first two alone, however, are true palisade layers, since their cells lie close together. The cells of the first



palisade layer attained a length of 70 microns. The cells of this layer are not all of the same length, some being considerably shorter than others, but they are all in close contact with one another. The second layer of cells consists of cells about one-half to three-quarters the length of the cells of the first layer. Numerous large intercellular spaces separate them into groups. Only occasionally is a cell of the third layer as much elongated as those of the second layer, and large intercellular spaces frequently separate the individual cells of this layer from their neighboring cells. The transition from the dark green to the light green is very abrupt, being only a matter of three cells, the first of which is as long a cell as that of one in the second palisade layer, and the next two are slightly shorter. Beneath the transition cells there is no second palisade layer; instead the cells below are cuboidal. The remaining cells are cuboidal throughout the light green area and the palisade cells appear very much like that shown in figure 11c for the leaf previously described.

Numerous air spaces are present between all the cells of the dark green area, with the exception of those in the first palisade layer, whereas in the yellow areas the cell spaces are smaller, and the layers of cells seem somewhat displaced, so that it is difficult to recognize sometimes to which layer a particular cell belongs. This also creates the appearance of one or two rows more than the normal seven or six in certain regions. However, I am quite certain that there has been no formation of extra layers in these regions.

Figures 12a, 12b, and 12c are of cells from the dark green region showing normal plastids with starch grains, and the fine cytoplasmic sheath which bounds the plastids. Figure 12b shows a cell from the second palisade layer, which contained a striated body, although from the dark green region of the leaf. Figures 12d and 12e are corresponding cells from a healthy mature leaf, and show that in reality the cells of the dark region, although appearing enormously elongated, never are as long as those in a healthy leaf of corresponding size.

8. *Histology of a mature diseased leaf of pattern type 4 upon a small pot-bound plant.* A leaf  $6.5 \times 3$  inches showing pattern type 4 (malformed, broad nervisequum) was taken from a very badly diseased and dwarfed plant which was only 8 inches tall,

pot-bound, and at least 6 months old. The leaf was very contorted, that is, its blade was twisted, its margins rolled under, and crenate. The material for fixation was cut out so that one-half of each piece consisted of tissue from the dark green region which, in the form of a sector of the leaf, occupied about one-third the area of the leaf, and tissue from the light green area adjacent. Each section then showed the histology of the yellow and the green areas.

The sections show the influence of the virus upon the dark green regions of such a diseased leaf when it begins to attack such healthy regions along their margins. The sections show the same histogenic stage of development in both the light and dark green areas. However the cell contents of the two areas are extremely different. The half of the sections belonging to the light area show the effects of the virus in the disintegration of the plastids, the presence of numerous striated bodies, and x-bodies. The cells of the first palisade layer of the dark green areas are 91 microns in length, and those of the second 54 microns in length, while those of the first palisade layer in the light green regions are about 79 microns, and those of the second 41 microns in length.

In the dark green areas large intercellular spaces are found between the lobed and rather small mesophyll cells. All the cells of this dark region are normal in appearance, with large rounded plastids nearly of the size of the nuclei. The latter appear small in comparison with the large size of the cells. Figure 13a shows a cell from the first palisade layer, and figure 13b one from the second. The material was not fixed at a suitable time for the appearance of starch grains, so that only a few are visible. The epidermal cells contain no plastids, and show only a thin cytoplasmic sheath bounding the wall, with the nucleus usually pressed against the lower wall.

Figure 13c shows a palisade cell from the yellow area. The plastids are much reduced in size and number. The nucleus in the figure is more or less surrounded by striated bodies. A single large vacuolated x-body is also more or less surrounded by striated bodies. The plastids appear as usual in the primordial utricle. Figure 13d shows a cell from the second palisade layer of the yellow green area. The nucleus is somewhat contorted, as it is pressed against the wall above the disorganized

striated body. A small x-body lies next to it. The plastids are very much reduced in size and number. The plastids measure only 6.8 microns in diameter (average of 50 measurements), while those of the dark green area measure 8.34 microns. Figure 13e shows the appearance of an epidermal cell from this area, with a swollen nucleus and disorganized striated bodies nearly filling the cell. An x-body, with two clearly defined vacuolated regions with dark rings around them, lies next to the nucleus. As these figures show, the presence of the x-bodies is by no means confined to young tissues only. Old diseased leaves like this will contain very many of them in the light green diseased areas, some of which are very large in size.

5. Histology of leaves showing type 5 pattern (pale, definitely blotched)

The sections were made from material obtained from a leaf  $5.5 \times 3.25$  inches in size and showing pattern type 4 (malformed, broad nervisequum) only at the tip of the leaf, while the rest of the blade showed the rounded 'cress green' blotches upon a 'light cress green' leaf, characteristic of type 5. Each piece of blade cut out for fixation was taken so as to include the darker green of a blotch, and the lighter green of the surrounding blade. Sections through the lighter areas show an under developed but clearly recognizable palisade layer, with air spaces irregularly distributed among the mesophyll cells. Usually the section at this point is only six layers thick. Through the darker areas the histogenic structure is practically the same, save that the cells appear more normal and their cell contents are decidedly normal. There are usually seven layers of cells present in the darker regions and well developed air spaces distributed evenly among the mesophyll cells.

Figure 14a shows the appearance of a palisade cell from the first layer in the darker green region, and figure 14b, a palisade cell from the lighter green region. As is clearly evident, the palisade cells from the lighter green regions are not as deformed or cuboidal as those in sections through the light green areas of leaves of type 4 (malformed, coarse nervisequum) described previously. The palisade cells in the lighter regions show a distinctly elongated form, though they are wider than normal palisade cells. The plastids are somewhat smaller.

Of course, as evident from the shades of green of the two areas, neither the lighter ('light cress green') or the darker areas ('cress green') are as green as is a healthy leaf. In figure 14b the nucleus lies in the primordial utricle, with an x-body beside it, and a striated body below it; another striated body lies on the opposite wall.

X-bodies and striated bodies are found throughout the leaf. They are found much more abundantly in the 'light cress green' areas. They are also found in the companion cells and phloem and xylem parenchyma of the small anastomosing veins.

6. Histology of leaves showing type 6 pattern (irregular, narrow nervisequum)

Pattern type 6 (irregular, narrow nervisequum) which appears later in the histology of the disease, and often lasts for a considerable period until the blooming of the plants, presents no new anatomical conditions. The leaves of this pattern show a double palisade layer in the dark green regions and six layers of cuboidal cells in the 'light cress green' regions, very much as described and figured for pattern types 3 (narrow nervisequum) and 4 (malformed, broad nervisequum).

IV. CYTOLOGICAL STUDY OF DISEASED AND HEALTHY CELLS

1. *The x-bodies in living cells and cells fixed with Schaudinn's solution*

In a previous paper (1924) I have described the appearance of the x-bodies associated with the mosaic disease of tobacco and *Solanum aculeatissimum*. In living cells the bodies are finely reticulate or granular and clearly visible often in great numbers in the hair and epidermal cells of diseased plants. One can study them with an oil immersion objective by simply stripping the epidermal tissue from the stem, midrib, or leaf blade of the living plant, and mounting it in water under a cover slip. The bodies in living cells are rounded, oval, or amoeboid in outline. They seem to change their form and position by the protrusion of small somewhat blunt pseudopod-like extensions of the body surface. In addition to this independent movement, they are carried along very slowly, as are also the nucleus and plastids, by the streaming cytoplasm

in the strands which stretch across the cell vacuole. I am giving here for comparison with the x-bodies in fixed material a figure from a photograph of a living cell in which the bounding layer of the x-body comes out very sharply (TEXT-FIG. 3). The figure is from a living hair cell from a light green region of a diseased leaf. This cell contains a great many of the bodies of varying sizes, four other smaller bodies being seen in the field slightly out of focus.

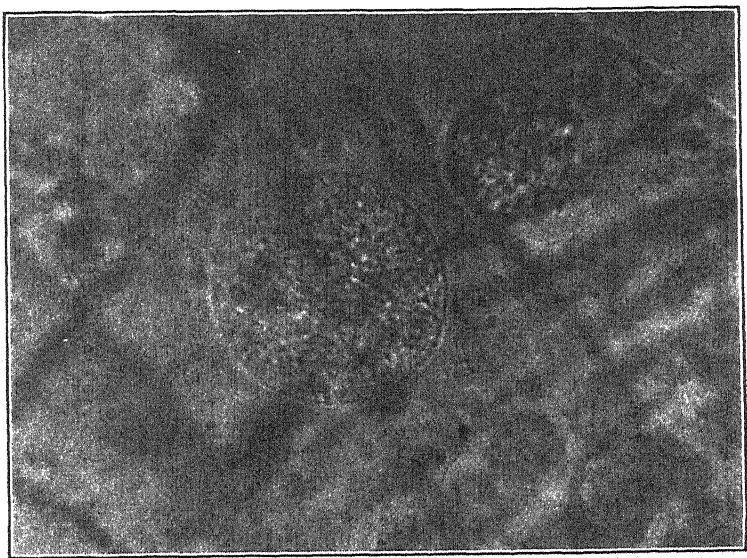


FIG. 3. A microphotograph of a portion of a living hair cell showing the presence of numerous large and small x-bodies. Taken with a  $\frac{1}{12}$  inch oil immersion objective.

An excellent method of fixation that I have used helps to bring out more clearly the protoplasmic nature of the x-bodies, and to distinguish them very effectively from the cytoplasm, cell nucleus, and other cell inclusions. A thin strip of epidermal tissue is removed from a diseased plant and fixed by dropping Schaudinn's solution upon it for several minutes, the additional drops preventing the solution from crystallizing. After several minutes, the solution is allowed to crystalize out and is finally removed when the material appears dry, with drops of 95 per cent alcohol. The material is run down into water, and then

immersed in iron alum for from three to five minutes. It is then washed again in water, and immersed in haematoxylin for a few minutes, washed again in water, and destained in 1 per cent iron alum under the microscope. The entire procedure is gone through with the strip of tissue lying on the slide, and the different solutions added with a pipette, and removed each time by absorption with a piece of cloth. When the material appears sufficiently destained the tissue is dehydrated with 95 per cent and absolute alcohols; clove oil is added, and then balsam. A permanent slide is thus secured in a very short time.

With such fixation, the x-bodies are found to be finely granular in structure, and distinctly protoplasmic in appearance, and clearly bounded and outlined against the cytoplasm of the cell. They occur as delicate amoeboid, rounded, or elongated bodies lying in the cytoplasm, and stain a very pale gray color. The striated crystals appear partially dissolved, that is the Schaudinn's solution makes them spread out so that they form dense black stained masses, but show no evidence of the lines of striation which are so clearly evident when the material has been fixed with the Flemming's solutions. The cell nuclei show the usual nuclear structures, black nucleole, and fine chromatin granules in the pale gray stained nuclear sap.

Figures 16a, b, c, and d, plate 26, show the appearance of the x-bodies when the leaf tissue is fixed in the manner just described. One of the x-bodies in figure 16a is distinctly amoeboid in form the other elongated. The cell contains three crystals, which were probably superimposed upon one another before fixation, and have spread apart, and appear only slightly dissolved. The nucleus appears homogeneous, and contains a single nucleole lying in a nucleolar vacuole. Four small plastids lie along the wall. Their starch grains appear as lens shaped clear areas. The x-bodies in figures 16b and 16d, each show one vacuole, within which a dark stained body lies, with radiating threads about it.

## *2. The x-bodies in fixed, sectioned, and stained material*

In preparations fixed with the Flemming's solutions or with Bouin's solution, and stained with Flemming's triple stain, the x-bodies appear as orange stained, lobed or amoeboid, rounded

or oval shaped bodies. They most commonly contain at least one vacuole, but often several or many vacuoles. The vacuoles are not all the same size in the body, and often they are so numerous as to give the body a distinct foam-like structure. The vacuoles often are distinctly bounded by a zone of dense granular material, and sometimes they contain red stained granules, about which are several delicate radiating threads. However, when a body contains several vacuoles, not every vacuole in the body will be bounded by a ring of granular matter, nor will every vacuole contain the red stained granules. In a few cases, however, I have observed such to be the case.

The x-bodies usually lie close to the nucleus, curved around it, pressed against it, and in many cases even producing a distinct indentation or hollow in its surface. However, the x-bodies may quite often be found lying in the primordial utricle or in the cell vacuole. On the whole the nuclei, although appearing swollen and enlarged in the cells of diseased areas, as shown by actual micrometer measurements and comparisons with nuclei of corresponding healthy cells, rarely appear distorted. The cells of diseased areas sometimes do contain amoeboid shaped nuclei. No doubt if the x-bodies exert pressure against the nucleus, distorting it, the nucleus can regain its original oval or rounded contour when the x-bodies move away, and the pressure upon the nuclear membrane is released. In such cases, where the nucleus in fixed and sectioned material appears indented, the x-body is usually present in the section pressing right into the region of the hollow or indentation.

The x-bodies often appear greatly elongated and attenuated at the center as if finally about to part in two. This suggests a simple division by constriction or fission. Such elongated bodies are often found in the youngest primordia where the cells are undergoing division. Figure 7, plate 29, shows a portion of a hair cell stained with the Flemming triple stain, containing a large nucleus with a single large nucleole stained red, and red stained chromatin granules strung along the delicate threads of linin; plastids lie about in the cytoplasm; a large x-body is present, containing large vacuoles marked by distinct rings of dense granular matter; the body appears to be pulling in two by means of a central constriction. Figure 8 of the same plate, shows several x-bodies present in a large hair cell;

the two smaller bodies may have originated from the larger body present in the manner suggested by the previous figure.

Figures 1 and 2, plate 27, are cells containing x-bodies whose vacuoles show the presence of small bodies within them. In figure 2, plate 28, the x-body contains two vacuoles, in each of which there is a dark staining body with delicate threads radiating from it. One of the x-bodies in the cell shown in figure 10, plate 28, also shows a similar vacuolar structure. In many cells x-bodies are found which contain vacuoles bounded by a ring of dense granular matter, within which a central granule with radiating threads is found. I have also observed lying in the primordial utricle small orange stained bodies, each containing a single vacuole in which a red stained granule with radiating threads is present. Such a cell is shown in figure 9, plate 29, from the epidermis of the light green region of a diseased leaf showing type 5 pattern (pale, definitely blotched).

### 3. *The striated bodies, tannin inclusions, and cuboidal bodies*

a. *Striated bodies.* I have described in the above noted paper, the presence in the diseased cells of large crystalline plates, which appear under the petrographic microscope to be decidedly crystalline in nature. These are exceedingly abundant in diseased tissue and are found only seldom in the dark green areas of diseased leaves. When in any tissue these crystals are present in large numbers, the x-bodies will also be found in great numbers in the cells. In basal plane, these crystals appear often as simple hexagonal plates, or very large irregularly shaped plates, which are often piled one above the other in several distinct layers. When viewed from the side they appear as oblong bodies, and show even in living material faint but distinct lines of striation across their short axis. Upon the addition of Flemming's solutions, as Iwanowski (1903) also noted, the crystals show this striation more distinctly, and often appear to be made up of distinct rods or needle crystals arranged side by side. In material fixed with Flemming's solution, and stained with Flemming's triple stain, the crystals usually appear as narrow bands of orange staining material. They lie along the cell walls or stretched across the cell vacuole. Often, due perhaps to overheating of the material during the process of imbedding and fixation, the crystals become more or



less disorganized and spread out across the cell vacuole. The individual striae in such cases appear much longer and somewhat separated and curved away from one another.

Iwanowski (1903) states that he was unable to dissolve the crystals. I have found that in the case of crystals in hair cells any strong acid or base when added drop by drop to the preparation under a cover slip, will finally cause them to spread out to such an extent that the striae disappear entirely, and a formless mass of material is left which retains its identity in the cell sap. The nucleus of the cell and the x-bodies retain their identity even after the breaking up of the crystals.

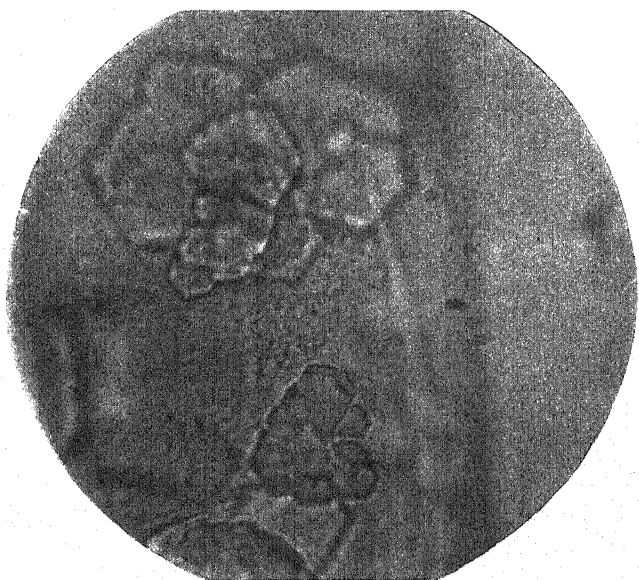


FIG. 4. A microphotograph of a portion of a hair cell showing the striated crystals in polar view (upper and lower center) and in side view (left). An amoeboid x-body with dark granules lies between and below the two crystal groups.

Such striated bodies have not been figured for other than Solanaceous plants. As noted above, Iwanowski (1903) has figured and described them in the diseased cells of tobacco. Rawlins and Johnson (1924) have also described and figured them in sectioned and fixed material from mosaic diseased tobacco plants. I have found them closely associated with the presence of disease in all tissues of tobacco plants and plants

of *Solanum aculeatissimum* (apple of Sodom) affected with the mosaic disease. They appear to be characteristic products of the reaction of the cells to the presence of the mosaic virus.

Text-figure 4 is a microphotograph of a living hair cell, taken with the oil immersion objective: a portion of the hair cell only is shown, containing several hexagonal crystalline plates, and one crystal at the left of the photograph is seen in side view. The hexagonal plates are superimposed upon one another in various positions. A bundle of much elongated raphides is also present in the cell lumen, but is quite out of focus. Between the two groups of crystals, and slightly below the plane in which they lie, an irregularly shaped x-body, with dark staining granules in its mass, is faintly visible.

b. *Tannin inclusions.* Tannin is found abundantly in the cells of growing points and flower buds, and often in the leaf primordia. It is apparently just as abundant in the growing points of healthy plants as in those of diseased plants. The tannin in these regions when fixed with the Flemming's solutions is in the form of globules or of finely granular material in the vacuoles of the cells. Often it is found as a dense network of dark orange staining material, such as Dangeard describes as occurring in the cells of the growing points and leaf primordia of various Gymnosperms.

Dangeard (1923) has described the formation of this tannin network. In the earliest formed vacuoles, constituting the 'vacuome' system of embryonic cells, the vacuoles contain only metachromatic granules which give the vacuolar network a black stain with haematoxylin. In the second phase, the vacuolar content now contains a yellow colored product which shows itself more and more distinctly. The vacuoles no longer stain deep black but appear indefinitely colored or brown or yellow. Later the vacuoles show a yellow tint, and the dark staining metachromatic bodies are confined to the borders of the vacuoles only. When the tannin appears in a cell it impregnates at the same time all the parts of the vacuolar system. His figures show the tannin deposits in the vacuome of young epidermal cells of leaf primordia of *Cedrus Libani*, and other Gymnosperms. Dangeard's figures 6, 7, and 9 of plate 10, and 11 and 12 of plate 12, of this tannin deposit, which he claims is a system of vacuoles in the form of a network, resemble closely those which I often

find in sections of the growing points of both healthy and mosaic diseased tobacco plants fixed with Bouin's solution, or Flemming's solutions.

In the cells of leaf primordia and the growing points of diseased plants in which the tannin occurs as a network, the x-bodies, striated bodies, and the cell nucleus can also be clearly seen in the cell. All stages of cell division, including progressive cell plate formation stages in which the ring of kinoplasmic fibers which are laying down the plate are seen in polar view, can be found in cells in which the central vacuole contains this network of tannin. If the tannin in this form really constituted a vacuolar system of narrow anastomosing tube-like vacuoles impregnated with tannin, the phragmoplast of a division figure, the cytoplasm, and the plastids ought to lie in definite positions with reference to this tanniferous vacuolar network, and certainly in such stages of cell division, this vacuolar network ought to show some signs of constriction or distribution to the two daughter cells being formed by the laying down of the cell plate. Certainly the cytoplasmic threads ought to envelope this network, and thus bound the vacuolar tubes, as cytoplasmic vacuolar membranes always bound cell vacuoles.

The evidence in my preparations, however, seems rather to point to the network of tannin being simply a deposit within the single cell vacuole, which itself is bounded by a distinct vacuolar membrane. The thin layer of cytoplasm which bounds the univacuolate cell of the growing stem region (ground meristem and protoderm) is still evident, as is its inner membrane which bounds the single large vacuole which nearly fills the cell. The cell nucleus lies in the primordial utricle or in the cytoplasmic threads which cross the vacuole, and the plastids lie along the threads or in the mass of cytoplasm around the nucleus. The striated bodies, and the x-bodies are found along the cell wall or in the cell space. In the cell lumen, sometimes completely filling it, or sometimes shrunken into a denser or closer meshwork, is the net-like deposit of orange or rusty brown colored tannin.

Only in the flower buds have I found the tannin deposited in the form of large vesicles which stain blue with the Flemming's triple stain. Tannin is extremely abundant in all parts of the young inflorescence as well as in the young flower buds. The

tannin vesicles are especially conspicuous in the cells of the floral envelopes and ovary walls. Here they often are so large that a single vesicle may nearly fill the entire vacuole. Such tannin vesicles are figured in cells of the floral envelope in plate 27, figures 4 and 5.

In unstained preparations of material fixed with Flemming's solutions, the tannin granules or globules are clearly differentiated from the x-bodies, since they are deeply blackened by the action of the osmic acid, while the x-bodies appear relatively colorless. The x-bodies stain pale orange with Flemming's triple stain. The tannic material, after bleaching with hydrogen peroxide for from one-half to one hour, loses its black color, and frequently stains a bright red with the Flemming triple stain when it occurs as globules or grains, purple when it is in the form of dense large tannin vesicles, and rusty yellow brown when it is deposited as a network in the vacuoles of the cells.

I have repeatedly applied microchemical tests for tannin (Haas and Hill, 1921) to the hair cells of diseased leaves containing the striated bodies and x-bodies, but have never found the slightest indication that either of these bodies is of the nature of tannin.

c. *Cuboidal bodies*. The brightly red staining minute cuboidal bodies found in the nuclei and in the promordial utricle of the cells of diseased tissue, as well as in the cells of healthy tissues, are not confused with the tannin bodies, which also stain bright red, since the latter are always rounded, and never cuboidal. The fact that they occur in the nuclei when the chromatin is sometimes evident in these as small red particles, even though the nucleus is in the resting condition, suggests they are of the nature of chromatin. Again they may appear in nuclei in which no nucleoles are present, which suggests they may be the nucleolar material in this cuboidal form. I have been unable to determine how they are distributed out into the cytoplasm, if their origin is really in the nucleus. They appear in my sections in nuclei which are evidently no longer able to divide. I find them most often in the nuclei of mature companion cells in the veins of the leaf blade, or in the large nuclei of the very large hair cells of mature leaves. When present outside of the nucleus, they lie along the primordial utricle, imbedded in the cytoplasm. I have not observed them lying free in the cell vacuole.

Figure 8, plate 29, shows a very large nucleus of a very large hair cell, in which, although a large nucleole occurs, several red stained cuboidal bodies are also present. Figure 14c, plate 25, shows the cuboidal bodies imbedded in the large oval nucleus of a mature companion cell from the vein of a leaf blade. There are no nucleoli present in this nucleus. Figure 8, plate 27, shows four of these cuboidal bodies lying within the cell nucleus. The cell is from the 'light cress green' region of a leaf showing type 5 pattern (pale, definitely blotched). Figure 9 of this same plate shows two cells from the parenchyma cells surrounding a small vein in a leaf blade, in which the cuboidal bodies are present in the primordial utricle. In the lower cell, the nucleus also contains two of these bodies.

These cuboidal bodies have been figured by Rawlins and Johnson (1924) who state that they are found in both healthy and diseased leaves, taking the black stain with Heidenhain's haematoxylin, and the bright red stain of the safranin of the triple stain.

#### 4. *The x-bodies in various tissues*

In fixed material the presence of these x-bodies always is associated with the other symptoms of the disease in a particular tissue. They are invariably present in the cells of the diseased areas, no matter what the nature of the tissues may be, or what their age, whether meristematic or mature. They are found in the youngest histogenic layers, dermatogen and periblem of the stem growing point, as well as in very young leaf and branch primordia which are just bulging outward from the growing point. Sections through the growing points of the stem, through leaves of all ages and sizes, through stems and roots, all show the presence of these intracellular bodies in very many tissues: dermatogen and periblem of the young leaf and branch primordia, and flower primordia; ground meristem and protoderm of the young stem and branch; epidermal, palisade, mesophyll, and vein tissues of the leaf blade; epidermal, parenchyma, phloem vessels, phloem and wood parenchyma of midribs, petioles, and stems; cortical, and phloem parenchyma of the root, as well as the dead epidermal cells, and xylem vessels of these organs.

a. *The x-bodies in the growing point.* The x-bodies are most

regularly found in the young leaf primordia just back of the growing point. In my sections of great numbers of growing points of diseased plants, I have only once found the x-bodies in the meristematic layers of the growing point itself. In that case, nearly every cell of the dermatogen and periblem layers contained one or several small oval, or elongated x-bodies with minute vacuoles within them. The two youngest primordia on either side of the growing point also contain the bodies in every cell of the dermatogen, and practically every cell of the periblem layers. However, the cells of the primordia being slightly larger than the cells at the point itself, and their nuclei comparatively smaller, the x-bodies are easily observed and appear distinctly larger in size, showing the presence of larger vacuoles within them. The x-bodies can be found in the cells as far as the tenth layer beneath the growing point, where, however, they are not nearly as numerous as at the point itself within the layers near the surface. The ground meristem cells below this tenth layer show no sign of the bodies or the striated bodies. The vein tissue is not present even in this region of the ground meristem. The presence of the x-bodies in the outer layers of the growing point, where there are as yet no conducting vessels, and the absence of the bodies in the cells of regions below the growing point where the conducting vessels are present, or in process of development, both seem to indicate that the disease travels easily along the epidermal and subepidermal tissues of the stem, through the young leaf primordia, and thus into the growing point itself. In one case in a series of sections of a growing point on which two young leaf primordia are just forming, x-bodies are found throughout the dermatogen cells of the primordia and in many of their periblem cells, up to where the dermatogen and periblem layers are just arising and curving up from the growing point itself to form these leaf primordia. The dermatogen cells and periblem cells along the growing point itself within these two young leaf primordia just bulging outward contain not a single x-body. One of the primordia is only about 40 microns in height, the other 1200 microns in height.

Figure 10, plate 29, shows a group of four dermatogen cells from the growing point of the stem itself. The cells, though belonging to the histogenic layers of the growing point, show the

presence of small vacuoles in their cytoplasm, and small vacuolate x-bodies are present in all four of the cells. In the case of the second and third cells the x-bodies lie adjacent to each other, and somewhat pressed against the opposite sides of the wall between the two cells.

The primordial histogenic layers of the stem, leaf, and branch are distinctly meristematic in nature and are regarded as not containing storage products, crystals, or reserve materials of any kind. Yet these cells may contain one, two, or more small vacuolate x-bodies, and less often the striated bodies. Figure 6, plate 18, shows a dermatogen cell from a very young leaf primordium in which a striated body lies along the outer wall of the cell, and a small oval vacuolate x-body is present. The cytoplasm appears as radiating strands about the nucleus.

b. *The x-bodies in the stem.* X-bodies and disorganized striated bodies are found in stems of diseased plants of various ages and thicknesses. The x-bodies are found in the epidermal cells, and usually in several layers of the collenchyma cells below the epidermis. Then the cells of the collenchyma, between these few outer layers and the region of the fibrovascular ring of the stem, contain no sign of the striated bodies or the x-bodies. The x-bodies are found in the fibrovascular bundles in the phloem tubes, and phloem and xylem parenchyma cells. The central pith cells again contain no bodies. Although the xylem tubes contain striated bodies, I have never observed the x-bodies in these vessels of the stem.

Figure 1, plate 27, shows an epidermal cell from a stem about  $3/8$  of an inch in diameter. The outer wall of the cell is very thick. A striated body lies against the inner wall. A large x-body containing small rounded vacuoles, in each of which a tiny red stained granule is present, lies next to the nucleus and extends out into the cell vacuole. Two oval plastids lie against the cell walls, one next to the nucleus, the other not far from the striated body. The clear rounded area in each represents a starch grain which has remained unstained.

c. *The x-bodies in the root.* X-bodies and striated bodies are found in sections of roots from diseased plants. These are found in the dead epidermal and collapsed cells around the root, which no longer contain nuclei or cytoplasm. They occur in the cortical parenchyma cells, and in the vein tissue within this

region. Figure 2, plate 27, is a cortical parenchyma cell from the root of a diseased tobacco plant, whose diameter was about  $1/16$  of an inch. The cell contains globules of tannin stained bright red with the safranin of the triple stain. A striated crystal lies on one side of the nucleus, which is distinctly pressed into a bean shaped form by the pressure of the large x-body down into it. Each vacuole of the x-body is distinctly surrounded by a ring of dense granular matter, and each contains a minute red stained body in its center. The appearance of the vacuoles with these bodies within them is very much like that of the small nuclei of an *Amoeba*. Figure 3 of plate 27 shows an x-body present in a cross section of a xylem vessel from a root. The nucleus appears to be in a somewhat degenerated condition, as shown by the condition of its chromatin masses and the absence of a nucleole. An oval x-body lies between the nucleus and the disorganized striated body.

d. *The x-bodies in the flowers.* My preparations of flower primordia and the young flower buds show cells so filled with tannin globules and vesicles that the nucleus and the tannin are the only distinguishable structures recognizable in these cells. However, in the floral envelopes, where the cells are somewhat larger, and the tannin is not so abundant, the cytoplasm and x-bodies can be found in these cells. I have not been able to find the x-bodies in the very small tannin filled cells of the ovules, nor in the clear spaces of the embryo sacs. This may simply mean that the floral material I have fixed thus far has not been penetrated by the virus, or that if the material had not been fixed at a time of day when the tannic metabolism is at its height, I might have been able to see more clearly the distribution of the x-bodies in the floral organs.

Although the seeds of tobacco are known not to carry the mosaic virus, there seems nothing present in the flower primordia to prevent the passage of the virus up into the meristematic layers of the primordia, as occurs in the growing point of the stem. No doubt ovules into which the virus does penetrate do not develop, while those which escape the virus do, and these form the seeds which are found in the mature seed pod. Further work on this point is necessary, in the hope of learning just what the effects of the virus are upon the embryo sac and the formation of the seed.



In figure 4 of plate 27, a small x-body lies in the bridge of cytoplasm beside the nucleus of the cell. Three small plastids are also imbedded in the cytoplasm beside the nucleus. A large tannin vesicle lies in the vacuole above the nucleus. In figure 5, plate 27, the x-body is pear shaped, and shows only a single small vacuole in it. It appears to be partially surrounding the nucleus. Two small plastids lie in the cytoplasm above the nucleus. Two large tannin vesicles are present. One lies in the vacuole, the other partially in the vacuole and across the cytoplasm.

e. *The x-bodies in the leaf.* As I have already described in the histogenic development of diseased leaves, x-bodies are found in leaves of all sizes and ages, from the earliest leaf primordia just appearing at the growing point, through all stages of leaf development. The bodies are also found in the vein tissues of the blade and in the various tissues of the midrib and petiole.

If the cells of the midrib are examined in a longitudinal section, the x-bodies can be found in nearly every cell of the epidermis, and the second, third, and fourth parenchyma layers inside of the epidermis. The bodies are small in the small epidermal cells, and appear larger and larger as they lie in the cells of the inner layers, which also are larger and larger with each successive layer further in from the epidermis. In the sixth and seventh layers, where the cells are very large, the x-bodies are also very large, and sometimes enormous. They are found only occasionally along the cells of these layers. X-bodies may be found, though very irregularly and infrequently, in the cells within the seventh up to the twenty-third layer from the epidermis. They are then found again in abundance in the phloem tubes, and companions cell, and phloem and xylem parenchyma cells of the two series of bundles or vascular strands found in the longitudinal section. The cells between the two vascular strands of the section do not contain them.

#### 5. *The x-bodies in dividing cells, and the method of their distribution*

My preparations of growing points and leaf primordia show every stage of nuclear and cell division and the distribution of the x-bodies between the daughter cells. I have figured all

stages of karyokinesis and cell plate formation, with the final distribution of the x-bodies in the daughter cells. Division figures in cells containing one, two, three, or more bodies are very common in the young primordia of leaves, and in the growing regions of the stem.

a. *The resting nucleus.* In cells in which the nucleus is in a resting condition, one or several bodies may be present. If the cell is univacuolate, the cytoplasmic strands will extend across the vacuole, and perhaps the nucleus will hang suspended in the vacuole by means of these threads. The plastids, if they are present, will lie in the cytoplasmic strands as well as in the primordial utricle. In one cell in which the nucleus appears in the resting condition,—that is, it contains a single large nucleole, and several small nucleoles, and the chromatin as fine granular matter—two large x-bodies lie close to one side of the nucleus, and another lies on the opposite side of the cell, but near the nucleus. In another cell in which the nucleus contains a single large nucleole, and the chromatin in the form of fine purple staining grains, two small oval vacuolate x-bodies lie along one side of the cell, both of the same size and form as if after a recent division. In the former cell, described above, the distribution of the several x-bodies in the cytoplasm suggests a chance distribution when the cell divides. In the latter cell described, the appearance of the two bodies suggests a previous multiplication on the part of the x-body, before the nucleus of the host cell has passed into the prophase stage as a preparation for karyokinesis (FIG. 1, PLATE 28).

b. *The prophases.* In the prophases, the nuclear content not only undergoes preparation for division, but the cytoplasmic make-up of the cell becomes considerably altered. The cytoplasm is more or less gathered about the nucleus, which may be suspended on a bridge of cytoplasm in the cell vacuole, or be supported there by radiating threads of cytoplasm. The x-bodies, if present, will lie either in the cell vacuole, or, as more generally is the case in the cytoplasmic mass around the nucleus. One cell in this stage of karyokinesis in my preparations contains a nucleus in which the chromatin is in the form of a continuous spireme, or very fine thread. The nucleole is still present. The plastids of the cell are gathered around the nucleus, as is also the cytoplasm. A single large vacuolated x-body lies out

in the cell vacuole. Another cell shows the nucleus with a thickened, but still continuous spireme. The plastids, are gathered around the nucleus in the cytoplasm. A large pear shaped x-body lies alongside the nucleus, as if in preparation for constriction. In another cell, whose nucleus is in the same condition, a very elongated x-body present in the cell also indicates preparation for constriction. Other cells with the nucleus at this stage show two or more x-bodies lying scattered about the nucleus in the vacuolar space, or imbedded in the cytoplasmic mass which surrounds it.

Figure 2, plate 28, shows a cell from a midrib region of a primordium, in which the nucleus contains a single large nucleole: the chromatin is still in the form of purple staining grains strung along the linin threads: a large vacuolate x-body is present. In figure 3, the chromatin of the nucleus is in the form of a thin spireme, and two vacuolate x-bodies lie near the nucleus.

At this stage, when the chromatin is in the form of a thin, spireme which is beginning to shorten and thicken, the fibrillar threads radiating around the nuclear membrane become evident. In vacuolated cells, the threads are often limited to the regions in which massive strands of cytoplasm cross the cell vacuole. These massive strands are caused by the slipping along the wall of the cytoplasmic strands which formerly crossed the cell vacuole, and which are coalescing in this manner, so that there may occur a cytoplasmic distribution to the daughter cells forming. In one cell, in which the radiating fibrillar threads lie about the nuclear membrane which encloses the thickened spireme, a small x-body lies pressed close to the nuclear membrane. In another cell (FIG. 4, PLATE 28) the small x-body lies above a cytoplasmic strand which still extends across the cell vacuole.

The fibrillar material evident in this radial stage swings around toward the regions of the nucleus which are destined to form the poles of the spindle, and in doing so the threads crossing each other form the multi-polar arc stage of the prophase. The chromatin is still in the form of a thickened and continuous spireme. In one cell, I have found a small x-body close to the nuclear membrane, while another lies against the cell wall. In the cell figured in figure 4, plate 28, there is found a small x-body lying close to the cell membrane.

In cells in which the chromatin is still in the form of a very much shortened and thickened spireme, the polar caps formed by the fusion of the fibrillar material are now present. The nuclear membrane is still present, but very thin, and nearly ready to disappear. In one cell, I have observed two small x-bodies, one lying next to each polar cap. In another cell, one small body lies next to a polar cap, while another large x-body lies below the other polar cap in the cell vacuole. Figure 6 shows a small vacuolate x-body in a cell whose nucleus is at this diarc stage of the prophase, with its spireme fragments shortened and thickened.

c. *Equatorial plate stage.* This disintegration of the nuclear membrane leaves the spireme fragments or chromosomes lying in the dense cytoplasmic mass which formerly surrounded the nucleus, while the spindle fibers move in from the poles to form the spindle. If there are any x-bodies present, they are usually found close to this chromosomal mass, and lie scattered around it in the cytoplasm. In one case, I observed one-half the chromosomal mass perfectly normal in appearance, while the other half, near which lay a large x-body, seemed to be still connected as if in the spireme stage, staining very dense red, and appearing much thinner and shrunken. More often there are two x-bodies found in cells showing the chromosomes arranged in the equatorial plate stage; and, seen in polar view, one x-body is usually found lying on one side of the chromosome group, while the other lies on the opposite side. In a cell in which the group of chromosomes appears in polar view, two large x-bodies lie on either side of the plate, which appears to extend diagonally between them (FIG. 8, PLATE 28). Very often, a side view of an equatorial plate stage, in which the chromosomes lie along the central region of the spindle, shows the several x-bodies lying near the poles of the spindle. In the case of the cell figured in figure 7, plate 28, two large bodies are present, one lying below the spindle, the other to the side of the division figure, suggesting a movement around to the opposite pole of the spindle.

d. *Anaphases.* In cells showing the chromosomes moving toward the pole, the x-bodies are usually found grouped about the spindle, or in the vacuolar regions above or below or to the side of the figure. An early anaphase stage in a cell shows one

immense x-body at one pole, while another lies below the lower pole. In such figures, it is often found that an x-body may lie entirely across the lower or the upper pole of the spindle, but whether this would prevent the daughter chromosomes from reaching the poles or not, is uncertain. The fibers, however, never appear distorted in any way by the presence of the bodies so close to them, even though in the section they appear to lie upon them. In the cell shown in figure 9, plate 28, the chromosomes are arranged upon the spindle in a late anaphase position: a large vacuolate x-body, whose vacuoles are clearly bounded by dense granular zones, and a small x-body lie near one pole of the spindle; two smaller vacuolate x-bodies lie above one pole of the spindle on the cytoplasm.

e. *The telophases.* This marks the completion of the nuclear division, and the distribution of the cytoplasmic threads to the daughter cells. The distribution of the x-bodies which lie in the cytoplasmic threads often appears very distinctly to be more than a matter of pure chance. In the telophase, when the spindle fibers are going to spread out across the cell, shorten and thicken, and their material become deposited along the central plane between the two daughter nuclei to form the cell plate, the cytoplasmic threads are also observed to be moving out across the cell and enveloping the phragmoplast fibers, which are moving further across the cell and becoming more and more separated from the daughter nuclei. The stretching out of the cytoplasm in this way also forces the two daughter nuclei down toward the newly formed cell plate, where they may more easily and directly influence the formation of new fibrillar material in the cytoplasmic boundaries of the phragmoplast fibers, which are being used up as the ring of kinoplasm moves across the cell. This arrangement of the cytoplasm in the form of a sheath about the daughter nuclei, with the phragoplast and cell plate between them, also provides for the mechanical distribution of the x-bodies. The cytoplasm containing the x-bodies is divided by the central cell plate, which is cutting it in two, and in this way the x-bodies come to lie in one or the other of the daughter cells. In addition to this chance method of distribution, there is some evidence pointing to some movement on the part of the x-bodies in controlling their own distribution, as the occurrence of x-bodies in the path of the moving cell

plate, their position near the spindle or the poles of the spindle, or along the forming cell plates. In one cell a distinctly constricted x-body lies beside a phragmoplast, as if one portion will be left in the upper daughter cell, while the other half after the constriction will be found in the lower daughter cell.

In an epidermal cell which is giving rise to a protrusion to form a hair cell, a division figure lies just in the region of the protrusion, that is, half way in the epidermal cell, and half way in the forming hair cell (FIG. 10, PLATE 28): the chromosomes are just reaching the poles; three x-bodies lie in the cell, one near each pole, and one to the side of the spindle. The position of the spindle indicates clearly that when each daughter nucleus is formed, one will lie in the epidermal cell, the other in the hair cell, and the x-bodies which lie near them will also be distributed in the same way. The central region of the spindle, in which the cell plate will form, lies in just the same plane, in which the cell plate must move across the cell to form a wall cutting off the hair cell from the epidermal cell. Alongside of the spindle, however, another large x-body is found, which will probably be in the way of the moving cell plate. It may move up into the hair cell, or back into the epidermal cell, when cell plate formation begins.

In another cell (FIG. 11, PLATE 28) the daughter chromosomes at the poles are massed closely together; the spindle is beginning to widen; a large vacuolate x-body lies below the pole of the spindle, and another x-body is seen in cross section near the upper pole of the spindle.

In another cell (FIG. 1, PLATE 29) the daughter chromosomes at the poles are beginning to loosen up and join to form a spireme; the phragmoplast has widened and is distinctly barrel shaped; an x-body lies near the spindle, and is seen in cross section as a narrow spindle shaped body with a small vacuole; a large vacuolate x-body lies below the division figure in the cell vacuole.

In another cell (FIG. 2, PLATE 29) the daughter chromosomes at the poles are joined to form a loose spireme; the phragmoplast has widened considerably, and shows the beginning of cell plate formation; three large x-bodies are present, one lying above the spindle in the upper half of the cell, while the other two lie below the spindle, and will be left in the lower daughter cell when cell division is completed.

In another cell (FIG. 3, PLATE 29) the chromatin of the daughter nuclei is still in the spireme condition, but each chromatin mass is now enclosed within a nuclear membrane; cell plate formation has been completed on one side of the cell, and the phragmoplast fibers on this side have disappeared and fused with the cell wall; the phragmoplast on the other side of the cell plate lies suspended in the cell vacuole, and has still to travel across the cell space; a large vacuolate x-body and two smaller univacuolate bodies lie in the upper vacuolar space; below that portion of the cell plate already laid down, a large x-body with two rounded vacuoles is present, and a small univacuolate x-body lies next to the nucleus.

An epidermal cell contains a cell plate figure suspended by cytoplasmic threads in the cell vacuole (FIG. 4, PLATE 29); the phragmoplast fibers are still present on both sides of the forming cell plate; the daughter nuclei are enclosed in membranes but show their chromatin still in the spireme stage; a large vacuolate x-body is present in the lower and upper halves of the cell, so that the cell plate will cut across the cell space, leaving one in each daughter cell; a third x-body is seen in cross section lying next to the cell wall, so that the cell plate will probably meet it and push it to one side or the other.

*f. Daughter cells.* When the cell plate has been formed across the cell space, and the phragmoplast fibers have fused with the cell membrane of the original cell, the two daughter cells appear also to be univacuolate, and show only a thin sheath of cytoplasm about the walls, while along the newly formed cell plate the cytoplasmic layer is very dense and wide, and the daughter nuclei lie here close to the cell plate. In such cells, the x-bodies are also found very close to the newly formed cell plate in the dense cytoplasmic mass. There however may be other x-bodies in the cell vacuole above or below the plate. As the cytoplasm now begins to draw away from this region, and swing up from the plate toward the farther walls of the daughter cells, cytoplasmic threads become evident across the cell vacuoles. The nucleus itself in each daughter cell is thus swung up into the cell space, and the x-bodies too may in this way be drawn up into the cell vacuole, and lie there in the vacuolar sap, or lie in the cytoplasmic strands as they move through the cell space.

In one case the cell plate has just been completed and the daughter nuclei are still found close to one another on opposite sides of the cell plate, staining very deep red, as their chromatin is still in the form of a spireme. One x-body lies in each cytoplasmic sheath along the cell plate. In another case, the cell plate is completed, and the daughter nuclei appear completely reconstructed, so that each shows a small nucleole present, and the chromatin in the form of purple staining grains again, after the dissolution of the spireme material. One daughter cell shows a large vacuolate body alongside the nucleus against the newly formed cell plate. The other cell shows two x-bodies, one against a wall of the cell and one below the daughter nucleus.

In a two celled hair in which a cell plate has just been laid across, the daughter nuclei are completely formed; the upper or tip cell contains two small x-bodies, the lower cell one x-body.

In the two daughter cells shown in figure 5, plate 29, the daughter nuclei seen in polar view are rounded; the chromatin has broken up into rounded purple staining grains strung along the linin threads; nucleoles are already present in each nucleus; the cytoplasm, by means of moving cytoplasmic threads, is just beginning to swing up from the newly formed cell plate and stretch across the cell space of each daughter cell towards the farther walls of the cells; a large vacuolate x-body is present in each cell.

Two daughter cells in figure 6, plate 29 show the daughter nuclei fully reconstructed, and their chromatin reduced to fine granules giving them an almost homogeneous appearance; each nucleus contains red stained nucleolar material; a large vacuolate x-body lies in the cytoplasm of each cell.

#### V. GENERAL DISCUSSION

I have classified and described both macroscopically and cytologically the commonly recognized symptoms of mosaic as a series of six patterns, each of which is correlated with the particular growth and histogenic stage of development which the leaf had reached when its cells were affected by the virus. The series is of course based on my studies of Connecticut Seed Leaf tobacco, inoculated with the particular strain of the virus with which I have worked. As noted above, I have already evidence of the existence of another or a modified strain of the virus which produces recognizable differences in the patterns.



The distinctness of the patterns, and their sequence have been repeatedly determined and confirmed by inoculating successive series of plants all similar in age, size and cultural conditions of growth, with virus obtained from the same diseased leaf, and in a corresponding leaf of the plant. The patterns described are: Type 1 (dark, vaguely blotched) found in the youngest of the large leaves on the plant at the time of the inoculation and above the inoculation point: Type 2a (pale crinkled) found in the youngest leaves present on the plant at the time of its inoculation, one of which will present the most distinct disease symptoms visible on the first appearance of the disease in the plant—'the critical leaf'; Type 2b (pale, vaguely blotched) resulting when leaves of type 2a (pale crinkled) expand in their further growth; Type 3 (narrow nervisequum) associated with the first leaves after the inoculation that developed from young primordia with the virus in them; Type 4 (malformed, broad nervisequum) found in the leaves which developed from the growing point of the stem when the symptoms of disease are already present in the plant, and the virus enters the young primordia as they are formed at the growing point; Type 5 (pale, definitely blotched); and finally Type 6 (irregular narrow nervisequum) which often persists until the flowering of the plant. It should be noted that this definite seriation in the symptoms is quite in agreement with the cyclic sequence of the symptoms in many diseases known to be caused by parasitic organisms.

As indicated in this seriation of the mosaic pattern types, the anatomical structure of a diseased leaf is correlated with the stage of its development when the virus entered it. When cuboidal cells are found in the light green areas of a given leaf, infection must have occurred at a very young stage and these cells have simply remained undifferentiated. The double palisade layer of the dark green areas, when studied from the developmental and histogenic standpoint, can be shown to be characteristic of young healthy green leaves at a certain stage of their development; neither has the hyperplastic development of a second layer of cells taken place, nor has hyperplasia of the cells themselves occurred in the dark green areas; the corresponding palisade cells of a leaf of mature development upon a mature plant may be much larger both in width and length

than I have ever found them to be in the dark green regions of diseased leaves.

The nuclei of the cells in the dark green area, as shown by the measurements given above, are always smaller than those of healthy cells in leaves of corresponding size. Although the dark green regions of young leaves first contain plastids larger in size than those in healthy young leaves of corresponding size, when the cells of older healthy and diseased leaves are compared, the plastids in healthy leaves are found to be larger than those in the dark green regions of the diseased leaves. It is repeatedly stated in the literature that both hyperplastic and hypoplastic areas occur in mosaic diseased leaves of tobacco, but so far as I find, the latter has not been regarded merely as arrested development.

In fact hypoplasia alone may be said to occur both in the light and dark green regions of the leaf blade of mosaic diseased leaves. Küster regards hypoplasia as leading to a condition in which 'the number, size, or differentiation of the cells of pathological tissues remains more or less below the normal.' The dark green areas then, since they represent a stage in the histogenesis of the leaf which is characteristic of a young healthy leaf, in reality present a case of retarded differentiation. Histogenic development has ended prematurely and the cells have simply elongated, but never to the extent found in healthy mature leaves. The dark green areas must also be regarded as showing hypoplasia.

The leaf patterns of many variegated plants resemble those found in the mosaic diseased leaves of tobacco. Such leaf patterns of *Coleus*, in which a red colored pigment is distributed only along the veins of the leaf, or in which the pigmented or yellow areas occur as large or small sectors of the leaf bounded by the leaf veins, or often crossing them irregularly, or as definitely or indefinitely bounded blotches, all suggest such patterns as are found in mosaic diseased tobacco plants.

Küster has made a careful study of many variegated plants and an especially fine study of the leaf patterns in *Coleus*. He believes that Baur's idea of a differential cell division is of extremely doubtful application, as far as the variegations in *Coleus* are concerned, since in the red areas there may be isolated cells or groups of cells which are green. The red occurring only

along the veins makes it appear that the cells in the vicinity of the veins receive from them something with which to build the anthocyanin. He favors the view that the patterns produced are very much in the nature of the precipitation phenomena described by Liesegang.

It is quite evident that, although Küster has shown in great detail that the striped and zebra patterns of many plants, and the blotching and mottling of others may be duplicated *in vitro* by different arrangements of the silver nitrate and the substratum, the initial cause which brings about the distribution of the active agent has still been left unsolved. What is there which operates within certain cells of the plant to first localize in centers and then produce the dispersion outward of material causing the rings, circular blotches, or irregular patches of color? The fundamental problem to be solved is the cause of the localized occurrence of the agents that bring about the changes in the leaves.

The mottling found in such leaf patterns of tobacco as types 1, 2, and 5, where the centers of the vein islets may conceivably be focal centers for the diffusion of toxic products, is possibly to be considered as the expression of Liesegang phenomena. The coarser blotching of type 5 (pale, definitely blotched) in the same way suggests perhaps that the virus has originated or centered at certain points and its effects are diffusing outward in all directions. However for such patterns as types 3, 4, and 6, in which the apparently healthy dark green blotches occur along the veins, while the entire leaf is decidedly in a diseased condition, as evident from its light green color and its simpler anatomical structure, the analogy is not so apparent.

In my opinion the dark green areas represent regions which have escaped infection in the early stages of the entrance of the virus into the leaf primordium. They are able to go through their histogenic development for a time, but soon the toxins of the virus hinder the completion of histogenesis, as far as differentiation is concerned. The cells continue to enlarge, and can only do so by elongating, since they are bounded by cells which, owing to the presence of the virus, are not enlarging to any extent, and are rather rigid. When the cells of the dark green regions divide, they are likewise under pressure from the surrounding light green regions which are enlarging more slowly,

so that a bulging upward to form a blistered or puckered region (savoying) results. It is not obvious just why the dark green areas are found along the veins of the leaf as they occur in the patterns 3 (narrow nervisequum), 4 (malformed, broad nervisequum), and 6 (irregular, narrow nervisequum). The occurrence of the red anthocyanin pigment along the veins of *Coleus* leaves is explained by Küster as due to the diffusion outward from the veins of the blade of products carried by the veins for the building of anthocyanin.

It seems scarcely possible that the veins should carry an anti-body whose effects are thus visible in the cells next to them which are first reached. The nervisequum patterns are found only in leaves which have developed as primordia from the growing point of the stem after the appearance of the disease symptoms in the plant. It seems to me more probable that the dark green regions represent areas into which the virus did not penetrate when the leaf was in the embryonic condition.

The different effects of the virus seen in the structure of the leaf blade can all be correlated with the influence of the virus upon the growth and differentiation of the blade during the early stages of the development of the leaf. The narrow blade, the frenched leaf, with irregularly lobed, crenate, and toothed margins, all indicate the dwarfing effects of the virus acting in the original leaf primordium. The various forms represent broadly the stages in histogenic differentiation at which the virus became effective, modified by accidents of distribution, degrees of virulence, etc.

The x-bodies appear to be distributed throughout the cells of diseased tissues in the youngest histogenic layers as well as in mature cells of old diseased leaves. My studies show, as indicated in plates 28 and 29, that in the growing points and in the formation of all new organs after the virus has reached the growing point, the distribution of the x-bodies is accomplished by the division of the infected host cells. Each new daughter cell formed contains before it is separated from its sister cell one or more x-bodies. I have some evidence that the x-bodies elongate and pull apart by constriction to form two, but as they are amoeboid in form, it is difficult to arrive at positive conclusions in such a matter.

I have found no evidence of the migration of x-bodies through

the cell walls in these young tissues, and there is no reason for assuming that it occurs since their spread into the young leaves, flowers, etc., is provided for by their distribution in the division of the host cells. The first infection of a healthy plant, and the migration of the virus to the growing points are of course another matter, the cytological aspects of which I am still studying.

As I have suggested in a previous paper (1924) the fact that the virus of tobacco mosaic is able to pass through bacterial filters does not mean necessarily that the size of the infective particles must be extremely minute. The x-bodies do not appear to be denser than the nuclei of cells which are known to be able to migrate through cell walls (Miehe, 1901; Schurhoff, 1906). I have repeatedly observed in my sections nuclei passing through minute, practically invisible openings of the cell walls. This generally occurs near the cut surface of the material, and is probably a wound response.

Kunkel (1918) describes the passage of the organism producing club root of cabbage, *Plasmodiophora Brassicae*, through the cell walls of affected plants. He states that the organism makes an opening in the wall, and believes he can see evidence of a softening of the wall. He states that after the organism has passed through, the cell walls appear practically unchanged.

The x-bodies are not disorganized nuclei. From my studies of sectioned material, I am convinced that binucleate and multinucleate cells do not exist in the healthy or diseased tissues of tobacco plants. As I have pointed out in a paper (1925) on polar views of division figures, the binucleate condition reported by Arber and Beer in the growing regions of a great many plants is based on a failure to recognize the existence of cross walls or cell plates that lie in or near the plane of the section.

The x-bodies are not tannin vesicles. They stain differently, and show an altogether different appearance from tannin vesicles or the tannin granules which are found in great abundance at certain periods in the cells of young tissues.

The striated bodies are evidently associated with the mosaic diseased condition of two Solanaceous plants, *Nicotiana Tabacum*, and *Solanum aculeatissimum*, and appear to be reaction products of the cell. I have not as yet been able to determine satisfactorily their chemical constituents, but am inclined to compare

them to cystoliths and similar cell inclusions of mixed organic and inorganic make-up.

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## Explanation of plates

## PLATE 18

The comparative sizes of leaves on a plant, which, because they have reached certain stages in histogenic development, will upon the inoculation of the plant present certain mosaic pattern types.  $\times 2/3$ .

FIG. 1 shows a leaf in the fifth stage of histogenesis which will show pattern type 1 (dark, vaguely blotched) if the plant becomes diseased.

FIGS. 2 and 3 are leaves which would show pattern type 2 (pale crinkled).

FIG. 4 shows a leaf which, upon the appearance of the disease in the plant, would show type 3 pattern (narrow nervisequum).

## PLATE 19

This leaf shows mosaic pattern type 1 (dark, vaguely blotched). Natural size.

## PLATE 20

FIG. 1. This leaf shows mosaic pattern type 2a (pale crinkled).

FIG. 2. This leaf shows mosaic pattern type 2b (pale, vaguely blotched). The crinkling shown in the earlier stage type 2a has entirely disappeared.  $\times .6$  approximately.

## PLATE 21

FIG. 1. This leaf shows mosaic pattern type 3 (narrow nervisequum).

FIGS. 2 and 3 show two stages in the development of a leaf showing pattern type 4 (malformed, broad nervisequum). Natural size.

## PLATE 22

FIG. 1. This leaf shows the presence of two consecutive leaf mosaic patterns; pattern type 4 (malformed, broad nervisequum) in the upper third of the leaf, and pattern type 5 (pale, definitely blotched) in the lower two-thirds of the leaf.

FIG. 2. This leaf shows pattern type 6 (irregular, narrow nervisequum).  $\times 2/3$  approximately.

## PLATE 23

The drawings were made with the aid of the Abbe camera lucida. A Zeiss microscope was used with  $1/12$  oil immersion objective and ocular 3. Figures 1-15, plates 23-26, have been reduced about one-sixth in reproduction, the present magnification being approximately 1020 diameters. Figure 16 and part of plate 27, done with the drawing board at a greater distance from the camera, have higher magnifications, as indicated.

FIGS. 1a-1c. Cells from a section of a leaf  $12 \times 6$  inches in size, showing pattern type 1 (dark vaguely blotched). The anatomical structure shows that histogenesis was at stage 5 at the time of infection.

Fig. 1a. A palisade cell showing numerous normal plastids along the cell walls. The nucleus is distorted by the pressure of an x-body against it. There is a single round vacuole in the x-body. Striated material lies above and to the side of the nucleus.

FIG. 1b. A cell from the third layer of the leaf (mesophyll). The striated material has dissolved out across the cell.

FIG. 1c. An epidermal cell showing an irregular shaped nucleus and a vacuolated x-body.

FIG. 2. A palisade cell from a leaf  $8.5 \times 3.75$  inches in size, showing pattern type 2a (pale crinkled). The cell appears normal save for the presence of two striated bodies which extend across the cell vacuole.

FIGS. 3a-3c. Cells from a diseased leaf  $10 \times 4.5$  inches in size, showing type 2b pattern (pale, vaguely blotched.)

FIG. 3a. A palisade cell. The plastids appear to possess vacuoles, which represent spaces left by starch grains. A striated body, and a large vacuolate x-body lie near the nucleus along the cytoplasmic bridge which extends across the cell.

FIG. 3b. A cell from the third layer of the leaf (mesophyll) showing the absence of a second layer of palisade cells in stage 5 of histogenesis.

FIG. 3c. An epidermal cell in which the cell nucleus, a vacuolate x-body, and a striated body are present.

FIG. 4. A palisade cell from a healthy leaf  $9 \times 5$  inches in size. There was only one palisade layer in this leaf, as is characteristic of leaves of vigorously growing plants when fully grown. The starch is present in the plastids as lens shaped grains of reddish blue (triple stain).

FIG. 5a-5d. Cells found in a section of a leaf  $5 \times 2$  inches in size, and showing type 3 pattern (narrow nervisequum).

FIG. 5a. A palisade cell of the first layer, and FIG. 5b a palisade cell from the second layer of the dark green region of leaf.

FIG. 5c. A palisade cell from the transition region between the dark green region and the yellow green region of the leaf.

FIG. 5d. Palisade cell from the light green area of the leaf. The x-body contains two vacuoles, in one of which is a bright red staining body with delicate radiating strands. The cell nucleus contains three nucleoles. The plastids appear normal in the primordial utricle. The small oval univacuolate bodies which lie on either side of the cell above the striated body are probably small x-bodies.

FIG. 6. Dermatogen cell from a primordium. A striated body lies along the outer wall of the cell. A small vacuolate x-body lies alongside of the large rounded nucleus characteristic of the cells of the histogenic layers.

FIG. 7a. Palisade cell from a very young primordium in which all of the cells showed disease symptoms. The large vacuolated x-body contains one vacuole in which a red stained body with radiating threads is present. I am doubtful as to the identity of the small vacuolated bodies lying about in the cytoplasm. A primordial cell of this size does not ordinarily contain plastids. It is probable that these are small x-bodies.

FIG. 7b. Palisade cell from a leaf primordium of a healthy plant. The nucleus still retains the characters of nuclei in meristematic layers in its large size and rounded form. The cytoplasm is typical for this stage in histogenesis when the cuboidal cells of the second layer from the upper surface are beginning to divide radially to produce the narrow cells of the palisade layer. The cells are multivacuolate at this stage.

FIGS. 8a-8c. Cells from a healthy leaf  $3 \text{ by } 5/8$  inches in size, and figs.

8d-8i cells from the dark and light green areas of a diseased leaf of the same size, showing disease pattern type 4 (malformed, broad nervisequum).

FIG. 8a. Palisade cell; FIG. 8b a cell from the third layer of cells in the section; and FIG. 8c an epidermal cell. This early stage in histogenesis (stage 4) is characterized by the formation of such narrow oblong cells as in fig. 8a, by the simple radial divisions taking place in the cuboidal cells of this layer. The cells are further characterized by the appearance of plastids, which are few in number, small, oval, and strung along the cytoplasmic strands traversing the cell vacuole.

#### PLATE 24

FIG. 8d. Palisade cell from the dark green region of the diseased leaf. FIG. 8e, a cell from the second palisade layer. FIG. 8f, a palisade cell from the second leaf layer (corresponding to a palisade cell shown in fig. 8d). FIG. 8g, a cell from the third cell layer of the section (corresponding to a second palisade cell shown in fig. 8e), of the light green region of the diseased leaf.

FIG. 8h. Epidermal cell from the dark green region of the diseased leaf. FIG. 8i an epidermal cell from the light green region of the diseased leaf.

FIGS. 9a-9d. Cells found in sections of leaves  $1 \times 3.75$  inches in size and showing type 4 (malformed, broad nervisequum) pattern. FIG. 9a, a palisade cell in a healthy leaf of this size. FIG. 9b, a corresponding palisade cell from the dark green region of the diseased leaf; it presents a much older cell as far as cell content is concerned than the cell figured in 9a from the healthy leaf. This cell, in spite of its small size, presents a mature type of structure such as would be found in a much larger healthy leaf; its plastids are arranged in the primordial utricle, and there is a single large vacuole present in the cell.

FIG. 9c. Palisade cell from the light green region of the diseased leaf. It shows the mature cell structure, although retaining the cuboidal form of a younger stage in histogenesis.

FIG. 9d. Epidermal cell from the light green region showing a striated body, the cell nucleus, and a vacuolate x-body in the cell vacuole.

FIGS. 10a-10c. Cells from a healthy leaf  $5 \times 2$  inches in size, and FIGS. 10d-10h, cells from a diseased leaf of the same size showing type 4 pattern (malformed broad nervisequum).

FIG. 10a. Palisade cell of the first palisade layer, and FIG. 10b, a cell of the second palisade layer. These cells illustrate the fourth stage in histogenic development as far as the cells of the second and third layers of the leaf are concerned, and the cell content of cells in this stage of development. There is a large central vacuole, the nucleus lies suspended in it by cytoplasmic threads, and the plastids lie in the primordial utricle which lines the walls. FIG. 10c. An epidermal cell in which vacuolization has continued so that a single vacuole is found, and the plastids have not increased in number.

FIG. 10d. Palisade cell of the first palisade layer in the dark green region of the diseased leaf. FIG. 10e, a palisade cell from the same region, belonging to the second layer of palisade cells. A comparison with the healthy cells figured in 10a and 10b shows that, although these cells present an identical picture as far as cell content is concerned, they are not as large as the cells of the healthy leaf.

FIG. 10f. Palisade cell which marks the transition from the dark green

region to the light green region of the diseased leaf; it contains a striated body, and a small x-body wrapped around the cell nucleus.

FIG. 10g. Palisade cell of the light green region of the diseased leaf; the nucleus is in the prophase stage; a crescent shaped x-body lies next to the nucleus.

FIG. 10h. Cell from the layer below this, corresponding to the cell figured in 10e of the second palisade layer in the dark green region. The cell nucleus and an x-body lie imbedded in a mass of disorganized striated material along the lower wall.

FIGS. 11a, 11b, and 11c. Drawings of cells from a mature leaf showing disease pattern type 4 (malformed, broad nervisequum).

FIG. 11a. Palisade cell of the first palisade layer in the dark green region of the leaf; FIG. 11c, a palisade cell from the second palisade layer of this region. FIG. 12c shows the cuboidal form of the cells that in the light green region correspond to a palisade cell of the dark green region such as is illustrated in FIG. 11a.

#### PLATE 25

FIG. 11d. Palisade cell from the first layer of the palisade cells in a healthy mature leaf; FIG. 11e, the form of a palisade cell in the second palisade layer of the same leaf.

FIGS. 12a-12c. Cells from a leaf  $6.5 \times 3.25$  inches in size, showing disease pattern 4 (malformed, broad nervisequum).

FIG. 12a. Palisade cell from the dark green area; FIG. 12b, a cell from the second palisade layer of the same area. FIG. 12c. A palisade cell which marks the transition from the dark green to the light green region. At this point there was no longer a second palisade layer.

FIGS. 12d and 12e. Corresponding cells of the palisade layers from a healthy leaf on an old mature plant. The cell in fig. 12d is more massive than the corresponding cell in the dark green area of the diseased leaf shown in fig. 12a. The plastids are also much larger.

FIG. 14c. Companion cell from the veins in a leaf blade showing pattern type 5 (pale, definitely blotched). The cell contains a large striated body, an x-body pressed against the wall, and a large oval nucleus; the cell nucleus contains red stained cuboidal bodies but no nucleoles.

#### PLATE 26

FIGS. 13a-13e. Cells from sections through a diseased leaf showing type 4 pattern (malformed, broad nervisequum).

FIG. 13a. Palisade cell from the first palisade region in the dark green region of the leaf; FIG. 13b, a palisade cell from the second palisade layer. The plastids appear large and rounded and contain no starch grains; the cell nuclei are small, in spite of the large size of the cells, as is characteristic of the cells in the dark green region of diseased leaves.

FIG. 13c. Palisade cell of the first palisade layer; FIG. 13d, a palisade cell from the second palisade layer of the light green region closely adjoining the dark green region, the palisade cells of which are shown in figs. 13a and 13b.

FIG. 13c. A very large vacuolate x-body lying in the cell vacuole, but somewhat obscured by the striated material lying above it; another striated crystal

lies along the wall, where the cell nucleus is also found. The plastids present quite a different picture from those in the cells of the dark green region: they are fewer in number and shrunken in size, and often barely discernible in the thin cytoplasmic sheath (primordial utricle) which lines the walls.

FIG. 13e. Epidermal cell from the light green region, showing abundant disorganized striated material, in which the individual striae are very distinct; an oval vacuolate x-body lies near the nucleus.

FIGS. 14a, 14b. Palisade cells from a diseased leaf showing type 5 pattern (pale, definitely blotched). FIG. 14a is from the slightly darker green region which marks the blotch. FIG. 14b is from the slightly lighter green region which surrounds the blotch.

FIGS. 15a, 15b. Palisade and mesophyll cells in an old yellowed leaf near the base of a diseased plant; the leaf had shown no signs of disease. The cells contain plastids in which the starch grains are stained red with the safranin, instead of having taken the normal blue of the gentian. Although striated bodies were present in all the cells, there were no x-bodies. Elongated needle-like crystals, or raphides, were found in the cell vacuoles.  $\times 1020$ .

FIGS. 16a, b, c, d. X-bodies drawn as they appear in a piece of epidermal tissue which was simply fixed with Schaudinn's solution and stained and mounted at once without sectioning. Magnification about 1615.

FIG. 16a. A portion of an epidermal cell of a midrib, showing two vacuolate x-bodies, one distinctly amoeboid in form. Four small plastids in which the starch grains appear as colorless areas are present. The nucleus with its single nucleole shows a homogeneous structure. The crystals are partially dissolved.

FIGS. 16b, c, and d show various forms of the x-bodies found in the cells, and their appearance with this mode of fixation and staining.

#### PLATE 27

FIG. 1. An epidermal cell from the stem of a diseased tobacco plant. The cell contains a large striated body, a dark stained nucleus, and an x-body in each of whose vacuoles a red stained body is present. The outer wall of the epidermal cell is very thick.  $\times 1020$ .

FIG. 2. A cell from the cortical parenchyma of a root of a diseased tobacco plant. The cell contains red stained globules of tannin. The nucleus is pressed in on one side by the x-body. The vacuoles of the x-body each contain a red staining minute body.  $\times 1020$ .

FIG. 3. A section through a xylem vessel in the root. The nucleus appears somewhat disorganized but still retains fragments of chromatin. The x-body lies between it and the striated body.  $\times 1020$ .

FIG. 4. A cell from the floral envelope of the flower. A large tannin vesicle is present, which nearly fills the upper vacuole of the cell. A small oval x-body lies in the bridge of cytoplasm supporting the nucleus in the cell space.  $\times 1785$ .

FIG. 5. A cell from the floral envelope of the flower. Two large tannin vesicles are present, one in the cell vacuole, and one in the cytoplasm but projecting somewhat out into the vacuole. A pear shaped x-body containing a single vacuole lies curved about the nucleus. A striated body extends along the lower wall of the cell.  $\times 1785$ .

FIG. 6. A xylem parenchyma cell from a cross section of the midrib of a large leaf showing type 1 pattern (dark, vaguely blotched). The nucleus appears somewhat degenerated. A large oval vacuolate x-body, whose vacuoles are clearly marked by rings of dense granular material, lies above the striated material in the cell. The thick walls of two adjacent xylem vessels are shown. The corners of the cell are very much thickened.  $\times 1785$ .

FIG. 7. A portion of a phloem tube of a vein in the blade of a leaf showing type 1 pattern (dark, vaguely blotched). A small vacuolate x-body lies above the elongated nucleus.  $\times 1020$ .

FIG. 8. A palisade cell from the light green region of a leaf showing type 5 pattern (pale, definitely blotched). Four cuboidal bodies lie in the nucleus which contains no nucleoles. An x-body lies next to the nucleus.  $\times 1785$ .

FIG. 9. Two parenchyma cells near the veins of the same leaf blade described above. The cuboidal bodies are lying in the primordial utricle, and, in the lower of the two cells, two of these bodies are lying within the nucleus.

#### PLATE 28

The drawings were made with a 2 mm. apochromatic oil immersion objective with compensating ocular 15; the original magnification of 1900 diameters has been reduced in reproduction to approximately 1290 diameters.

FIG. 1. A dermatogen cell from a leaf primordium, showing the nucleus in the resting condition, and the presence of two small vacuolate x-bodies.

FIG. 2. A cell from the midrib of a leaf primordium in which the nucleus is in a resting condition, and a single very large x-body lies near the nucleus.

FIG. 3. A cell from the ground meristem of a branch primordium. The chromatin is arranged in a thin spireme. Two vacuolate x-bodies are present in the cell.

FIG. 4. A dermatogen cell from a young leaf primordium in which the nucleus contains a thickened spireme, and radiating cytoplasmic threads are present about the nucleus. A vacuolate x-body is seen in section lying in the cytoplasm.

FIG. 5. A cell from the midrib region of a primordium. The radiating threads have folded over to form a multipolar arc. The spireme is thickened, but entire. An x-body cut by an oblique section is seen near the cell wall.

FIG. 6. A cell from the midrib region of a leaf, showing the diarc stage of the fibers, or polar caps. A small x-body lies in the vacuole.

FIG. 7. An epidermal cell of the midrib. The chromosomes are arranged at the equator of the spindle. Two large vacuolate x-bodies are present.

FIG. 8. A cell from the ground meristem of the stem. The chromosomes lie in the equatorial plate, but the section has cut them somewhat diagonally, distorting the polar view. Two vacuolate x-bodies lie in the cytoplasm.

FIG. 9. An epidermal cell from the midrib. The chromosomes are passing to the poles. A large vacuolate x-body lies near one pole, and several smaller x-bodies lie in the cytoplasm.

FIG. 10. An epidermal cell of a leaf from which a hair is just protruding outward. The chromosomes have just reached the poles. Three vacuolate x-bodies are present.

FIG. 11. A cell from the midrib region of a leaf primordium. The daughter



chromosomes at the poles are gathered together in tight knots. Two x-bodies are present in the cell, one near each pole. One body is cut diagonally and lies near the wall.

## PLATE 29

FIG. 1. An epidermal cell from the midrib of a leaf primordium. The chromosomes at the poles are loosening from the knot. The spindle is beginning to widen. Two x-bodies are present. The one next to the spindle is seen in cross section.

FIG. 2. An epidermal cell of the midrib of a leaf primordium. The chromosomes are still joined in a loose knot. The spindle has widened out to a greater extent, and is distinctly barrel shaped. Three vacuolate x-bodies are present, one near the upper daughter nucleus, and two below the division figure.

FIG. 3. An epidermal cell of the midrib of a leaf. The phragmoplast has reached one wall and disappeared, but is still traveling across the cell vacuole on the other side. One large x-body with several smaller ones lies in the upper portion of the cell. A second large x-body with another small univacuolate body is found in the lower cell space.

FIG. 4. An epidermal cell in which the division figure lies suspended in the center of the cell space. Three x-bodies are present, one above and one below the forming cell plate, and one against the wall in the direct path of the approaching phragmoplast.

FIG. 5. Two daughter cells, after a recent division of a protoderm cell of the growing point of a stem. A vacuolate x-body is present in each cell.

FIG. 6. Two daughter cells after division of a cell in the ground meristem of the growing point of the stem. A large vacuolate x-body is present in each cell.

FIG. 7. A portion of a hair cell in which the large vacuolate x-body seems to be dividing by constriction.

FIG. 8. A portion of a hair cell containing a large nucleus, in which, in addition to the large nucleole present, small cuboidal bodies are also present. One large and two smaller vacuolate x-bodies lie in the cytoplasm below the nucleus.

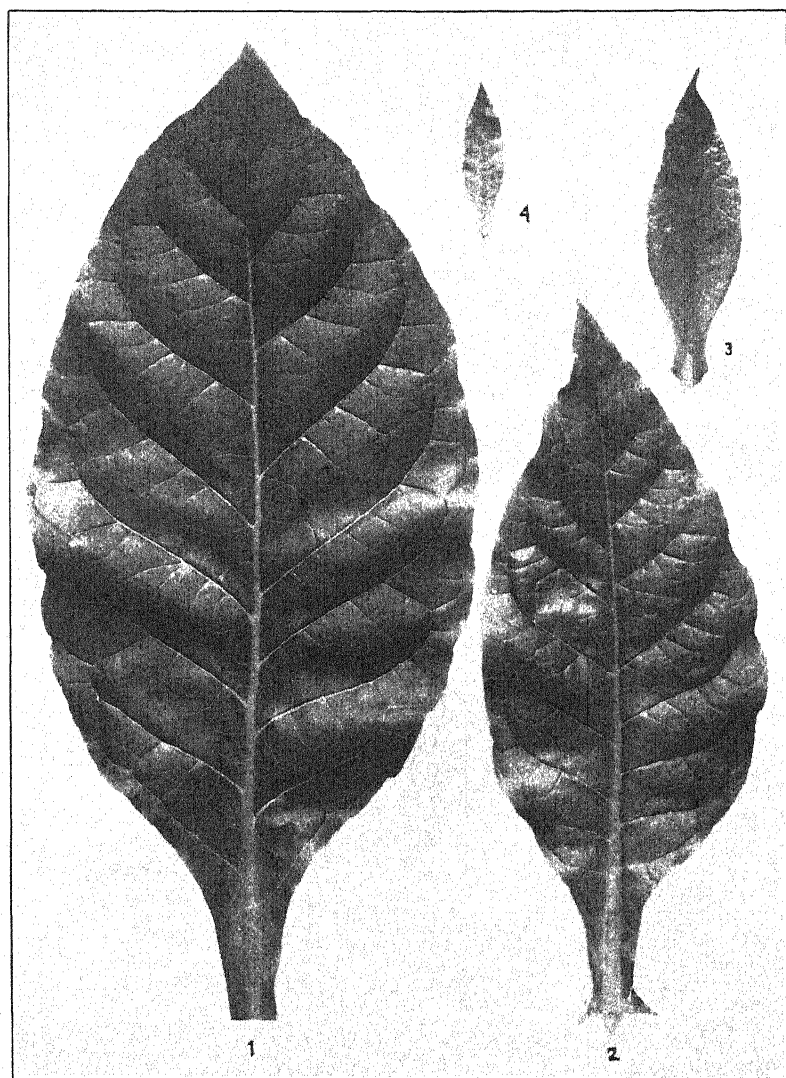
FIG. 9. An epidermal cell from a diseased leaf showing type 5 pattern (pale, definitely blotched). The x-body contains many vacuoles bounded by rings of dense granular matter, and each contains a minute red stained body with delicate threads radiating outward. Small bodies lying in the cytoplasm show a similar structure within their vacuoles.

FIG. 10. A set of four cells from the dermatogen of the growing point of the stem, in which an x-body is present in each cell.

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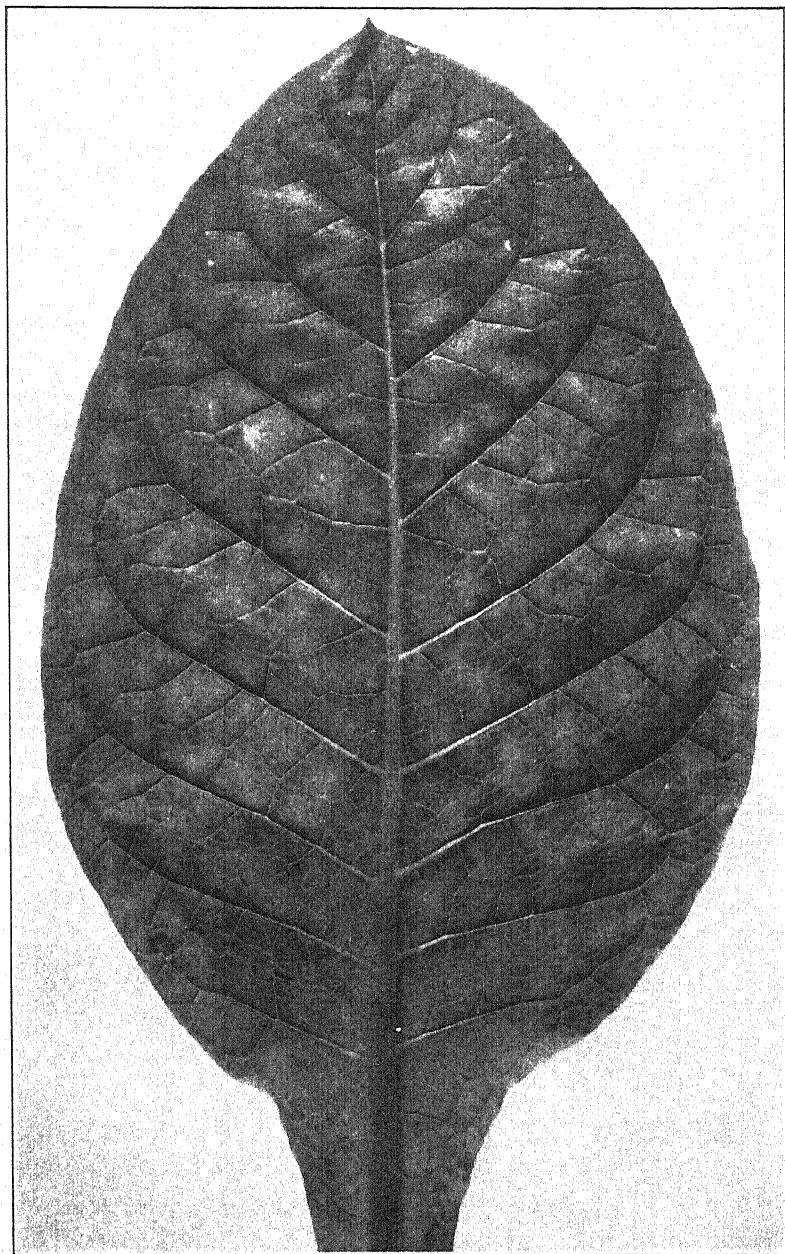
Plates 23-29 were made by the Photo-Gelatine Printing Company,  
Hoboken, N. J.





Relative sizes of leaves acquiring certain mosaic patterns upon infection





Mosaic pattern 1 (dark, vaguely blotched)



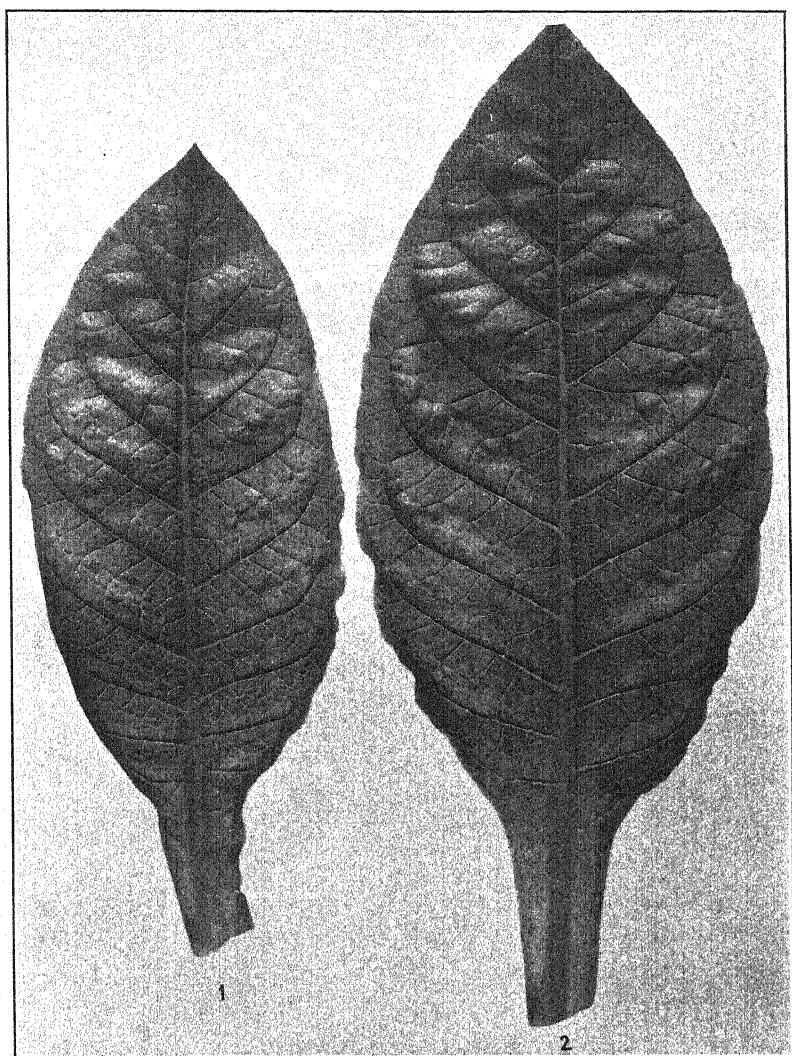


FIG. 1. Mosaic pattern 2a (pale crinkled)

FIG. 2. Pattern 2b (pale, vaguely blotched)





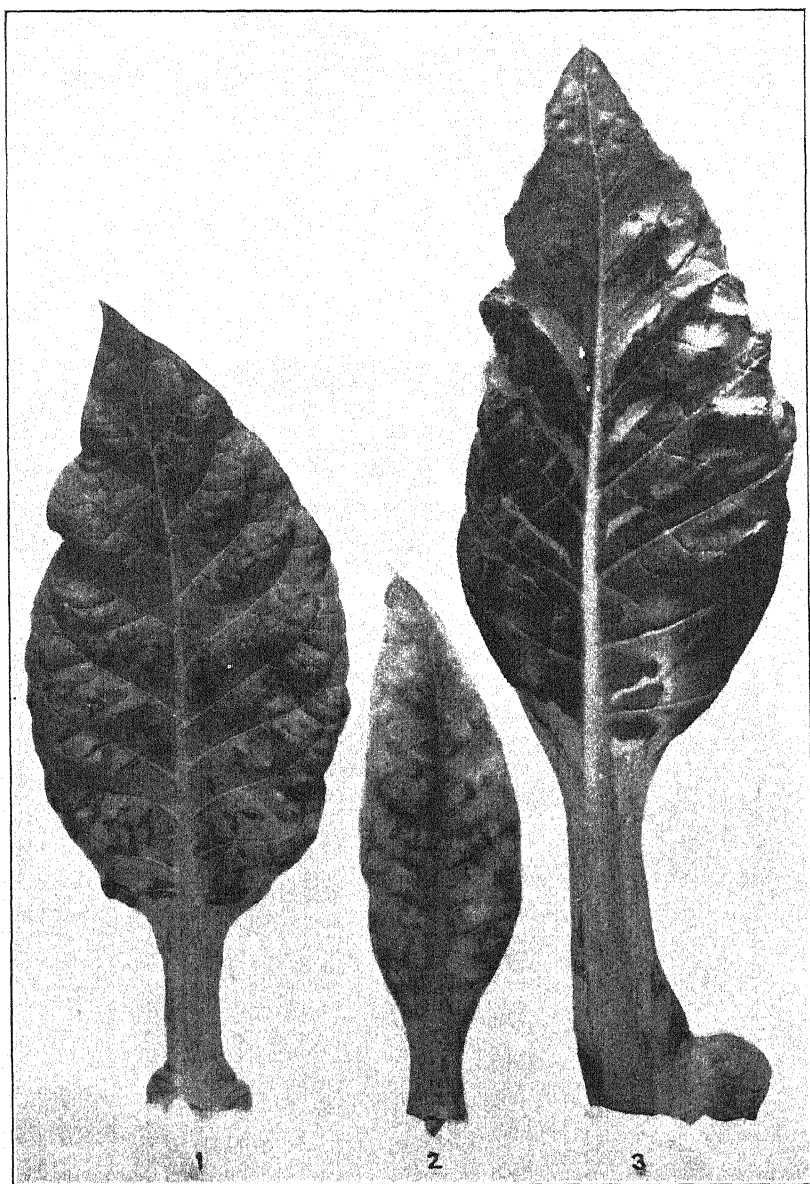


FIG. 1. Mosaic pattern 3 (narrow nervisequum)  
FIGS. 2, 3. Pattern 4 (malformed, broad nervisequum)



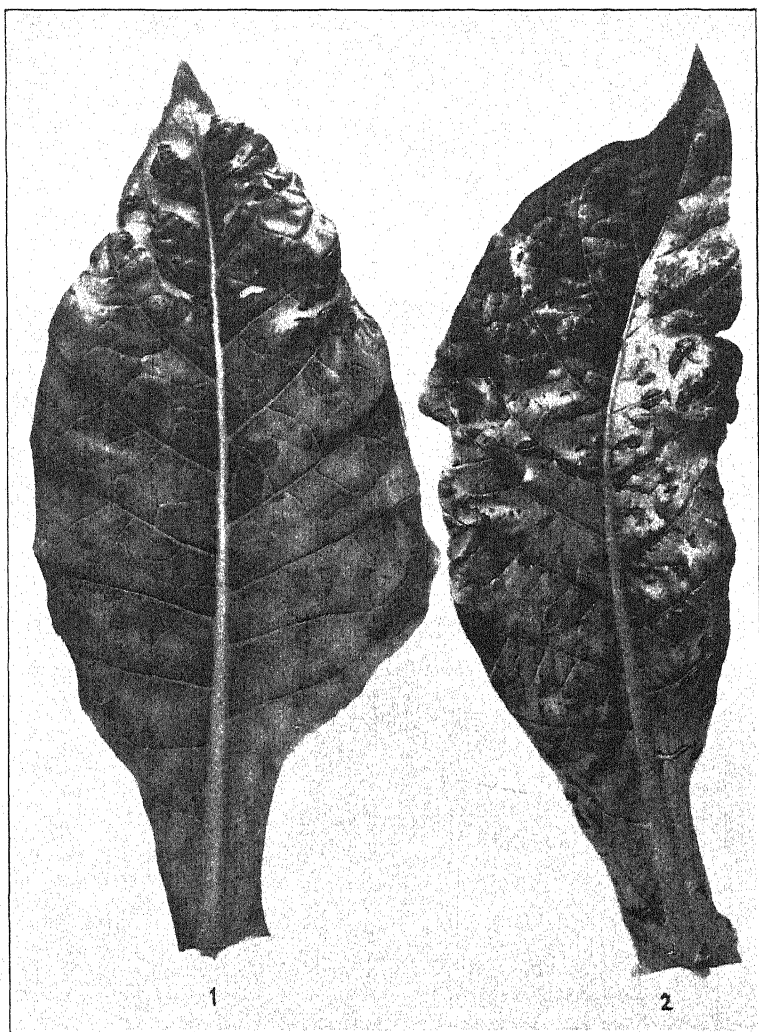
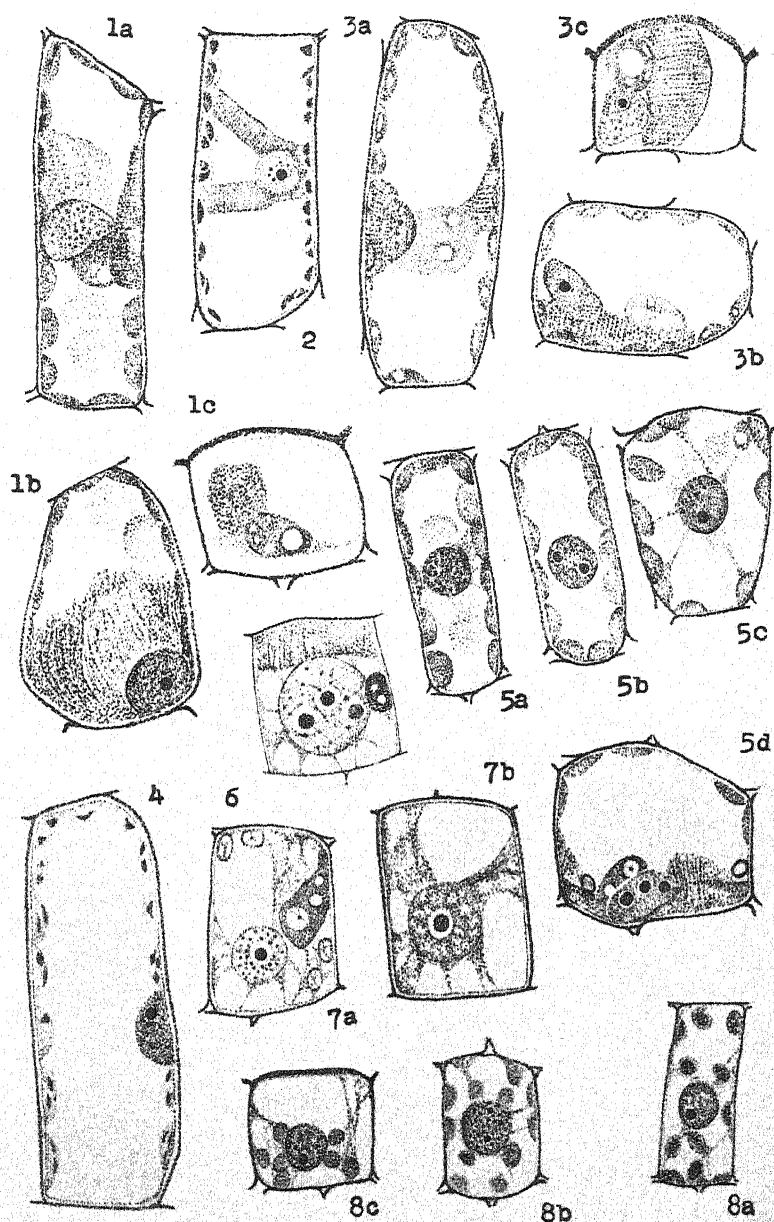
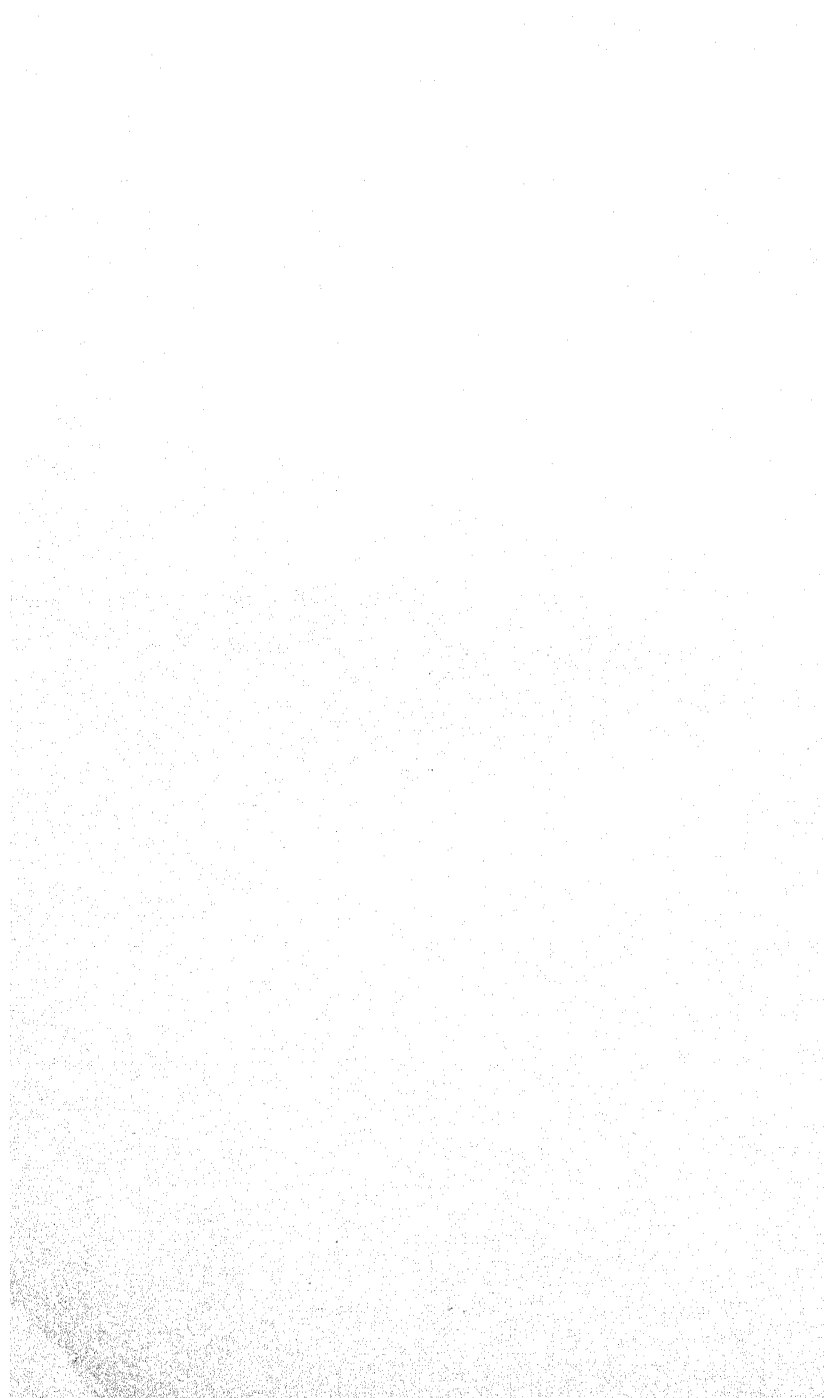


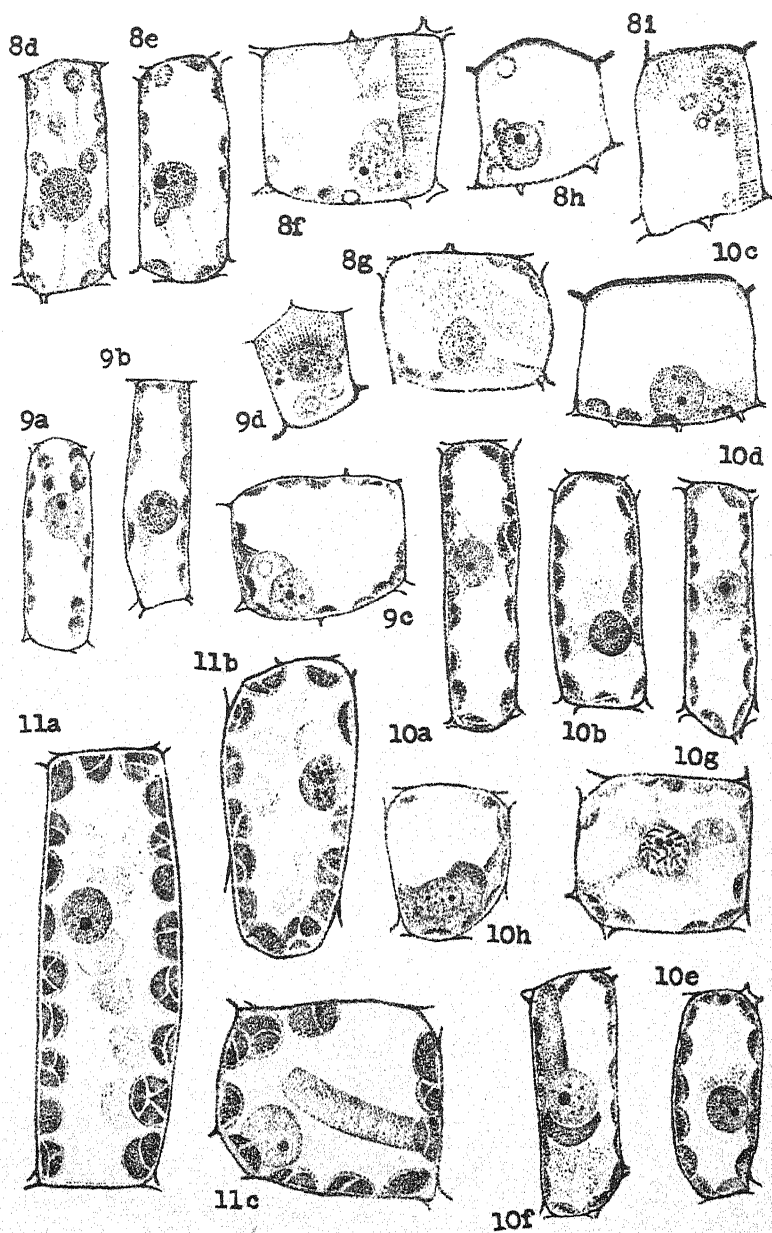
FIG. 1. Mosaic pattern 4 (malformed, broad nervisequum) above; below pattern 5 pale definitely blotched)

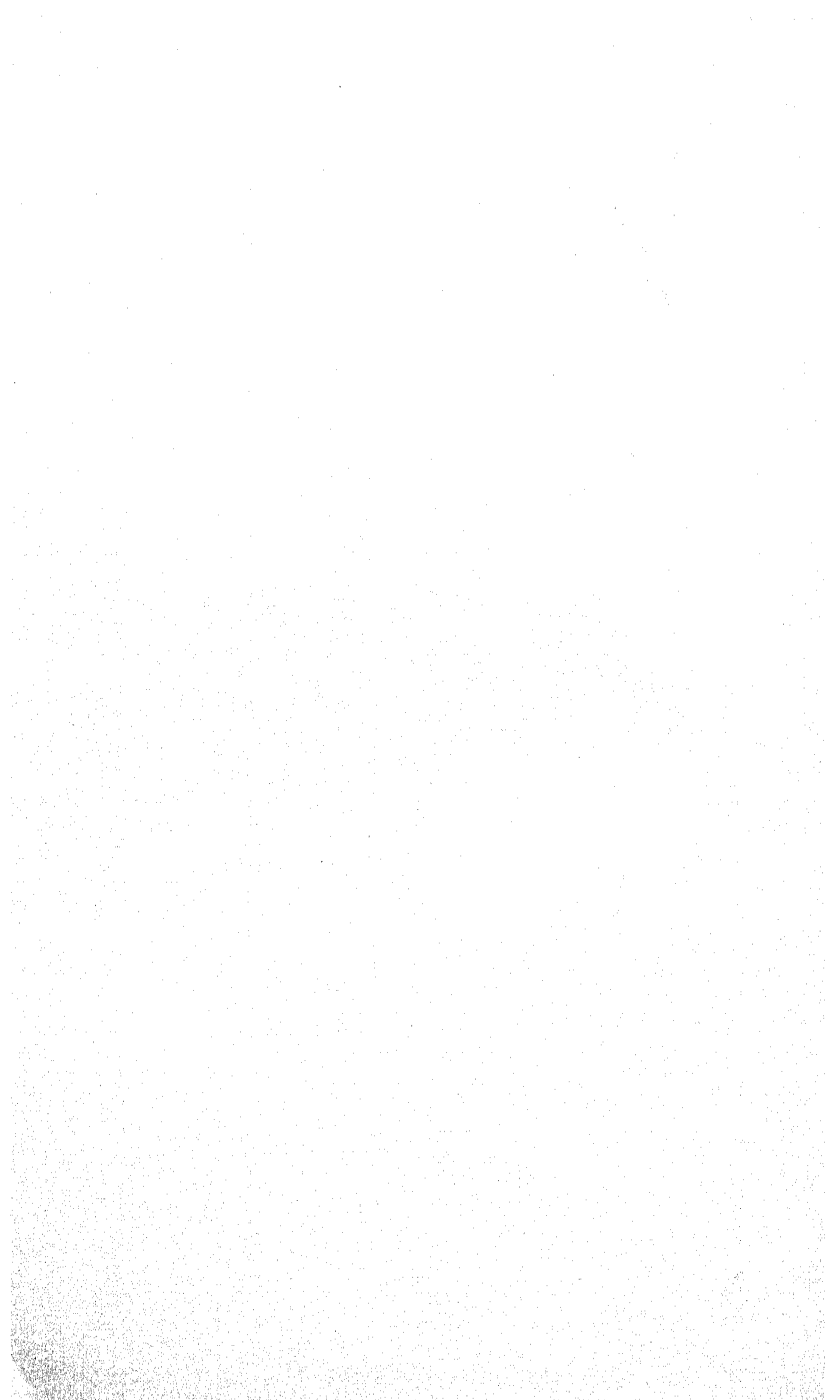
FIG. 2. Pattern 6 (irregular, narrow nervisequum)



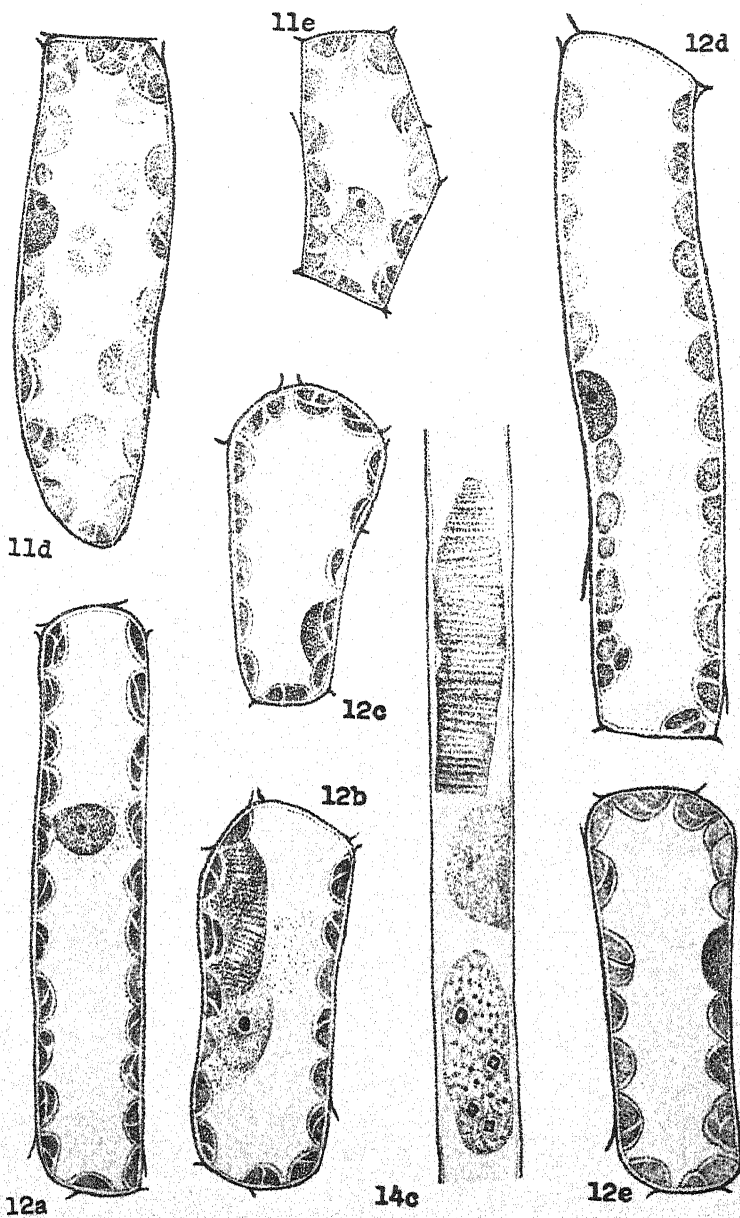










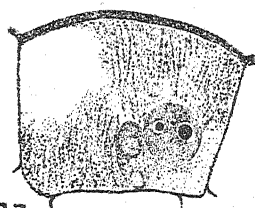




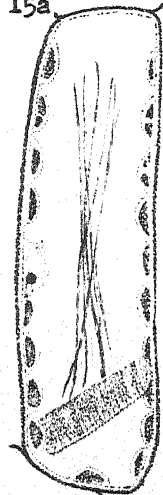
14a



13e



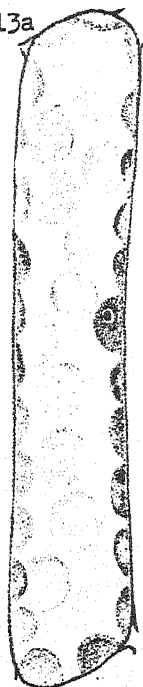
15a



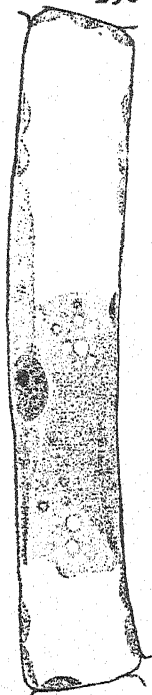
14b



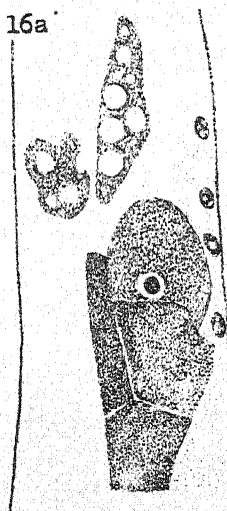
13a



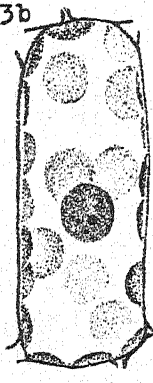
13c



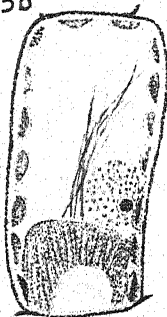
16a



13b



15b



16c



13d



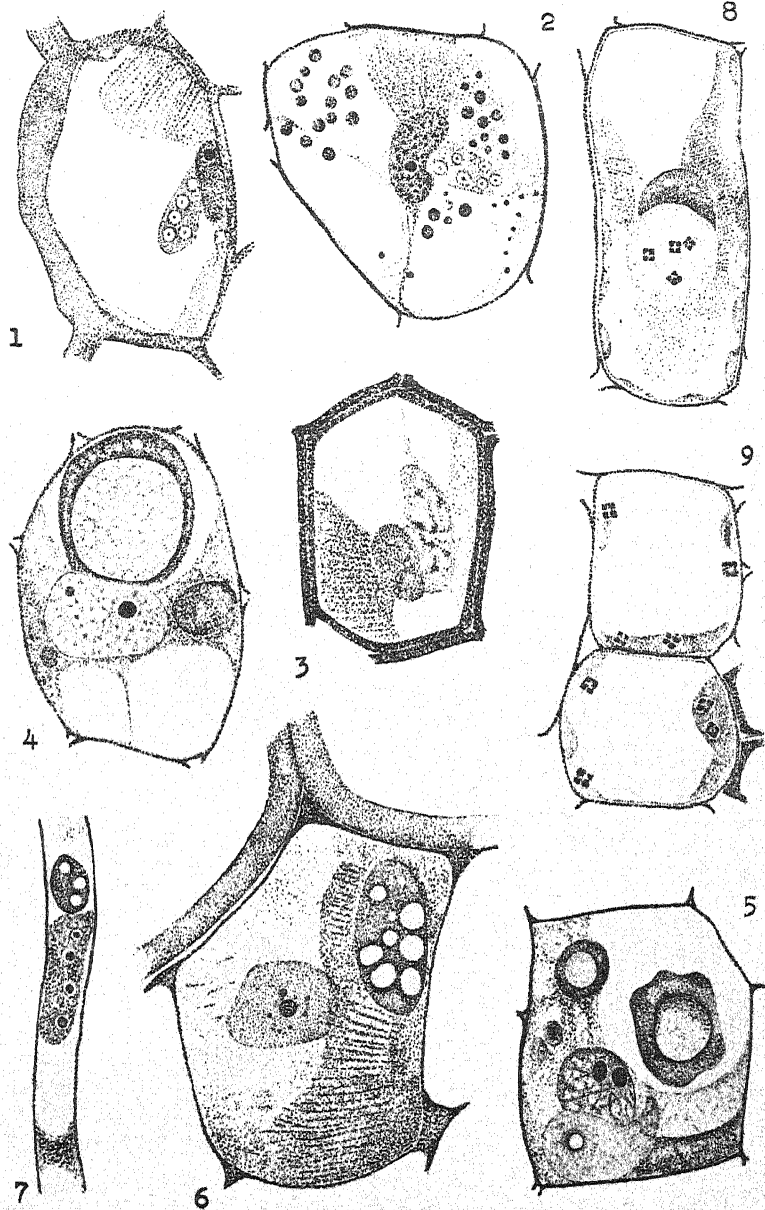
16b

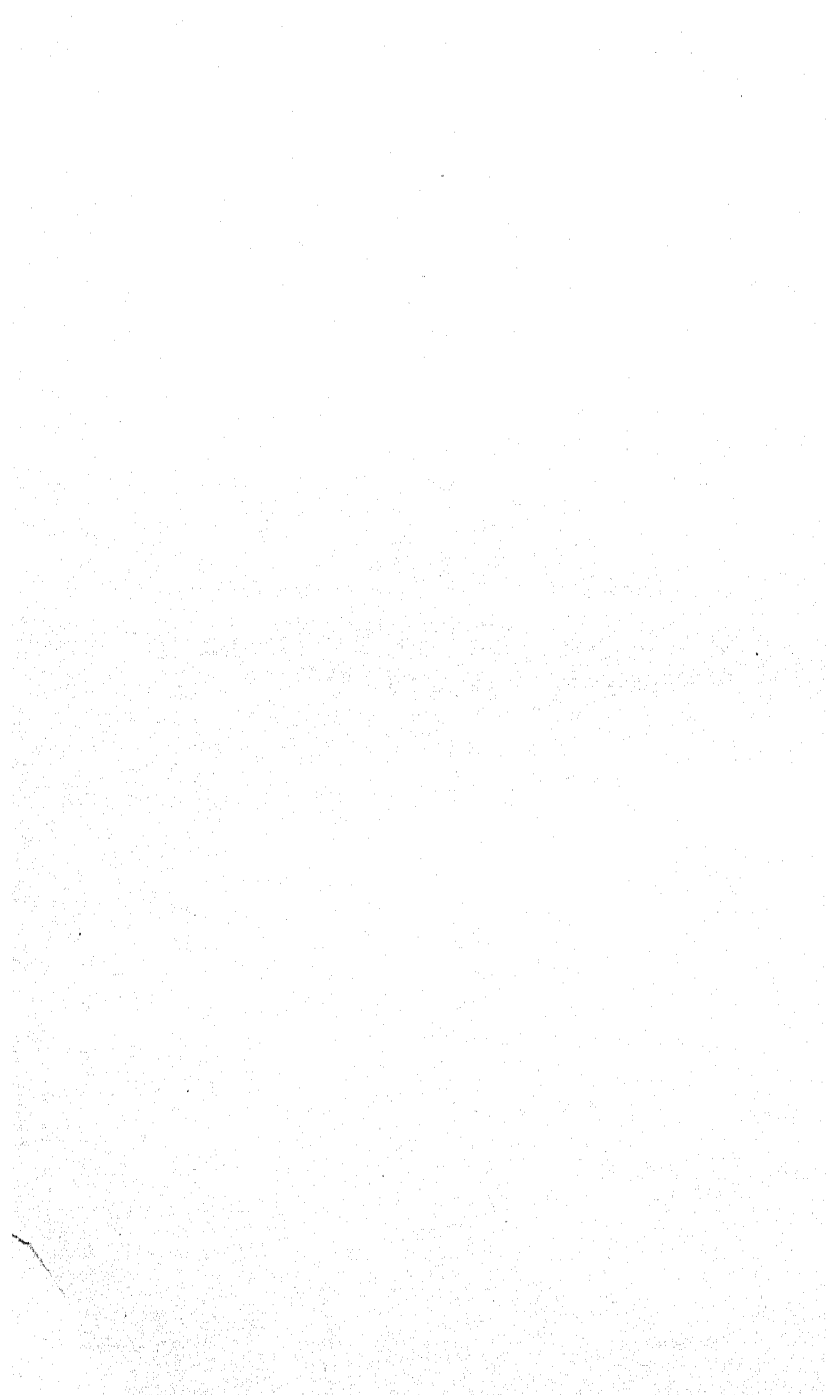


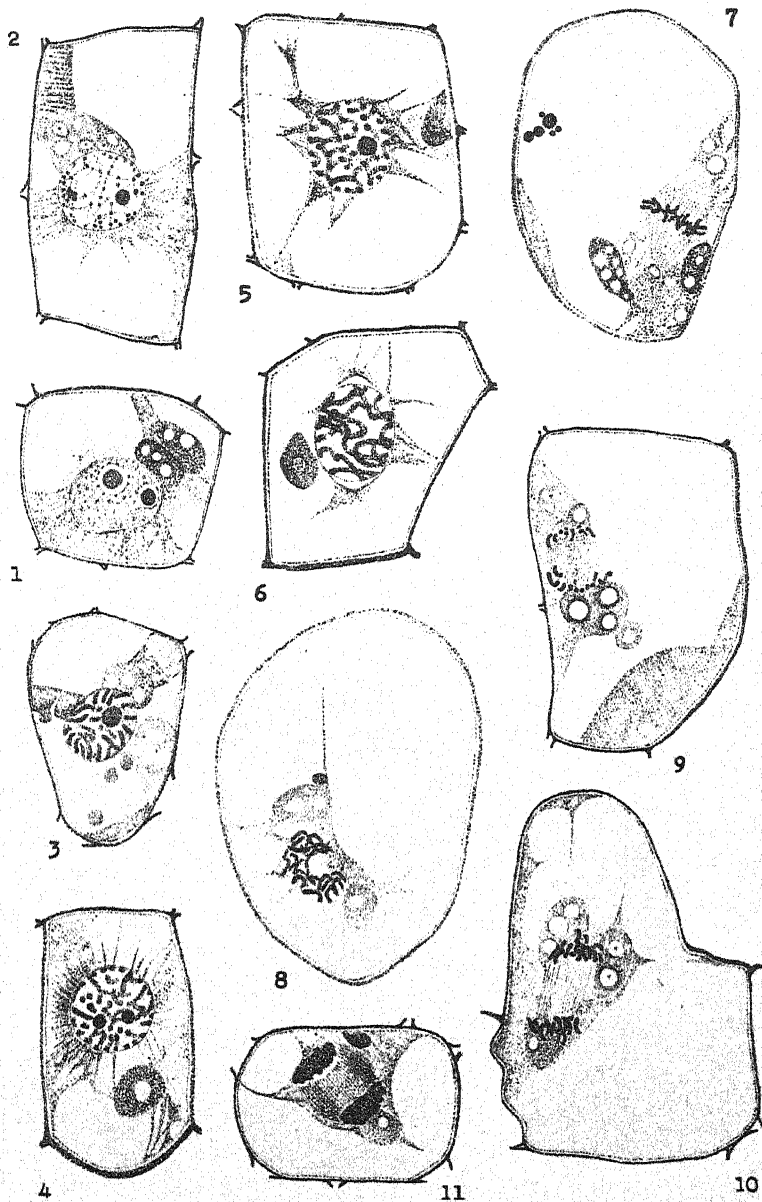
16d





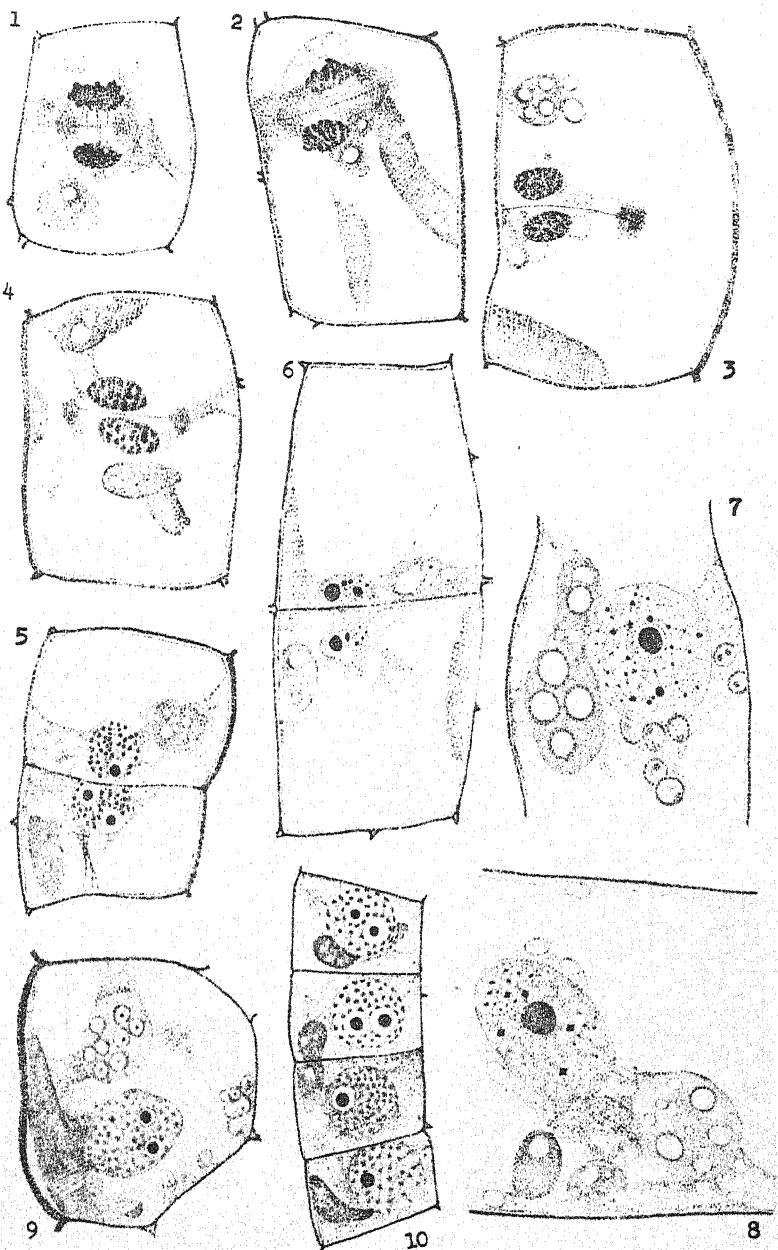














## The identity of 'Cuspa' (Conoria ? Cuspa H. B. K.)

S. F. BLAKE

In 1825 Humboldt, Bonpland, and Kunth described under the name *Conoria* ? *Cuspa* a tree which had been collected by Humboldt and Bonpland near Cumaná and Bordones in north-eastern Venezuela. The short mention there made of the uses of the tree for timber and particularly in medicine, as a febrifuge, is derived from the much fuller account given earlier by Humboldt,<sup>1</sup> which is of sufficient interest to quote in full.

Le *Cuspa*, assez commun dans les environs de Cumana et de Bordones, est un arbre encore inconnu aux botanistes de l'Europe. Il n'a servi pendant long-temps qu'à la construction des maisons, et il est devenu célèbre, depuis l'année 1797, sous le nom de Cascarilla ou Quinquina de la Nouvelle-Andalousie. Son tronc s'élève à peine à quinze ou vingt pieds de hauteur; ses feuilles alternes<sup>2</sup> sont lisses, entières et ovales. Son écorce, très-mince et d'un jaune pâle, est éminemment fébrifuge; elle a même plus d'amertume que l'écorce des véritables Cinchona, mais cette amertume est moins désagréable. Le *Cuspa* s'administre, avec le plus grand succès, en extrait alcoolique et en infusion aqueuse, tant dans les fièvres intermittentes que dans les fièvres malignes. Le gouverneur de Cumana, M. d'Empan, en a envoyé une quantité considérable aux médecins de Cadix; et d'après des renseignemens donnés depuis peu par Don Pedro Franco, pharmacien de l'hôpital militaire de Cumana, le *Cuspa* a été reconnu en Europe presque aussi bon que le Quinquina de Santa-Fe. On prétend que, pris en poudre, il a l'avantage, sur ce dernier, d'irriter moins l'estomac des malades, dont le système gastrique est très-affoibli.

Sur les côtes de la Nouvelle-Andalousie, le *Cuspa* est regardé comme une espèce de Cinchona; et l'on assure que des moines aragonois, qui avoient résidé long-temps dans le royaume de la Nouvelle-Grenade, ont reconnu cet arbre par la ressemblance de ses feuilles avec celles des véritables Quinquinas. Cette assertion n'a rien d'exact; c'est justement par la disposition de

<sup>1</sup> Humboldt, Voyage aux régions équinoxiales du nouveau continent, fait en 1799, 1800, 1801, 1802, 1803, et 1804, par Al. de Humboldt et A. Bonpland. 1: 366-367. Paris, 1814. The title cited in the Nova Genera et Species, 'Relat [ion] hist [orique]' is carried in the volume at the base of the first page of each signature, but not on the title page.

<sup>2</sup> "Vers le sommet des branches, les feuilles sont quelquefois opposées, mais constamment dépourvues de stipules."

ses feuilles et par l'absence des stipules, que le *Cuspa* diffère totalement des plantes de la famille des Rubiacées. Il se rapproche peut-être de la famille des Chèvre-Feuilles ou Caprifoliacées, dont une section a des feuilles alternes, et parmi lesquels on trouve déjà plusieurs Cornouillers remarquables par leurs propriétés fébrifuges.

Le goût à la fois amer et astringent et la couleur fauve de l'écorce ont pu seuls conduire à la découverte de la vertu fébrifuge du *Cuspa*. Comme il fleurit à la fin de novembre, nous ne l'avons pas trouvé en fleur, et nous ignorons à quel genre il appartient. Depuis plusieurs années, j'ai demandé vainement à nos amis de Cumana des échantillons de la fleur et du fruit. J'espère que la détermination botanique du *Quinquina* de la Nouvelle-Andalousie fixera un jour l'attention des voyageurs qui visiteront ces régions après nous, et qu'ils ne confondront pas malgré l'analogie des noms, le *Cuspa* avec le *Cuspare*.

The specimens of the 'cuspa' tree collected by Humboldt and Bonpland were in young bud only, and were described by Kunth as a new species doubtfully referred to the violaceous genus *Conoria*, which is now universally recognized as a synonym of *Rinorea*. In Humboldt's account the tree was said to reach a height of barely 15 or 20 feet, but Kunth, by some error, described it as 'arbor maxima (teste Bonpl.)'. Since Humboldt's time nothing more has been learned about the identity of the 'cuspa.' Eichler,<sup>3</sup> after examining original specimens, excluded the species from the Violaceae, but on account of the very immature condition of the flower buds was unable to assign it to any family. Baillon,<sup>4</sup> however, shortly afterward referred to it as *Rinorea Cuspa* in a notice of the useful plants of the Violaceae. In the present writer's recent revision of the American species of *Rinorea*,<sup>5</sup> *R. Cuspa* was placed, following Eichler, among the excluded species.

During the summer of 1925, I was able to examine the type of *Conoria* ? *Cuspa* preserved in the Humboldt and Bonpland Herbarium at Paris. It proves to be a species of *Aspidosperma*, of the Apocynaceae, and is identical with the plant described several years ago as *Aspidosperma lucentivenium* Blake. The tree is common in the coastal region of Venezuela, from the Paraguana Peninsula east to Cristóbal Colon, and occurs also on the island of Trinidad. Dr. N. L. Britton some time ago

<sup>3</sup> In Mart. Fl. Bras. 13<sup>1</sup>: 388. 1871.

<sup>4</sup> Hist. Pl. 4: 346. 1873.

<sup>5</sup> Contr. U. S. Nat. Herb. 20: 518. 1924.

called my attention to the probable identity of *A. lucentivenium* with *A. sessiliflorum* Muell.-Arg., described from Trinidad in 1859-60. A sheet of the latter without flowers collected at Pointe Gourde, Trinidad, by N. L. Britton and W. E. Broadway (no. 2648), is in the National Herbarium, and Dr. Britton has sent a flowering specimen (in bud) collected at Camaronaro, Trinidad, by J. Dannouse (no. 4979). Study of these specimens, in connection with Mueller's original description, leaves no doubt as to the identity of the Trinidad species with that of the mainland.

The labels of the 18 sheets of this species from Venezuela and Trinidad examined in the National Herbarium contain no notes as to the uses of the tree, and only one collection (*Curran & Haman 840*, from La Guaira) bears a vernacular name, 'amargoso.' Mr. Henry Pittier, of Caracas, informs me that the bark and leaves are still employed in the preparation of a febrifugal drink. The tree is generally known as 'amargoso,' and in the region of Barcelona as 'cuspa.' The name 'cuspa' is more commonly used for *Cusparia trifoliata* (Willd.) Engler (Rutaceae), from which is derived the well known 'Angostura bitters.' The *Cusparia* is also known as 'cuspare,' 'cascarilla,' and 'quina de Nueva Andalucia.' Apparently the vernacular names of these two trees, both of which have febrifugal properties, are used somewhat indiscriminately, for Humboldt, who particularly distinguished the 'cuspa' (*Aspidosperma Cuspa*) from the 'cuspare' (*Cusparia trifoliata*) in the quotation given above, applied the name 'quinquina de la Nouvelle-Andalousie' to the 'cuspa.'

Material sent by Mr. Pittier has been given a preliminary chemical examination by Mr. O. F. Black, of the Bureau of Plant Industry, U. S. Department of Agriculture. Both the leaves and bark, especially the latter, contain an amorphous alkaloid. The dried bark yields about 2.5% by weight of crude alkaloid, insoluble in water but soluble in alcohol, chloroform, and dilute acid. It appears to be unstable in the air. Mr. Black is continuing his investigation of this alkaloid with a view to the publication of his findings in the Journal of the American Pharmaceutical Association.

*Aspidosperma Cuspa* is related to *A. Vargasii* A. DC., another species of the coastal region of Venezuela, but is readily

distinguished from it by the leaves, flowers, and fruit. In *A. Cuspa* the corollas are glabrous outside, the pods (including the stipe) are 2.8–3.5 cm. long and 1–1.5 cm. wide, the leaves are rounded or emarginate to obtuse at apex, and the petioles are 3–7 mm. long. In *A. Vargasii* the corollas are densely pubescent outside, the pods (including the stipe) are about 6.5 cm. long and 2.5–3 cm. wide, the leaves are often or usually acute or acuminate, and the petioles are 7–16 mm. long. The two species differ in habit, also, according to Mr. Pittier's observations, *A. Cuspa* being branched from the base and smooth-barked, while *A. Vargasii* has a naked trunk and flat crown, and its bark is rimose. The synonymy of *A. Cuspa* is as follows:

***Aspidosperma Cuspa* (H. B. K.) Blake<sup>6</sup>**

*Conoria* ? *Cuspa* H. B. K. Nov. Gen. & Sp. 7: 242. 1825 (type from Bordones and Cumaná, Venezuela).

*Aspidosperma sessiliflorum* Muell.-Arg. Linnaea 20: 399. 1859–60 (type from Trinidad, Sieber 53).

*Aspidosperma lucentivenium* Blake, Contr. Gray Herb. 53: 46. 1918 (type from between LaGuaira and Rio Grande, Venezuela, Curran & Haman 970).

<sup>6</sup> This designation, attributed to the present writer, has been employed by Mr. Pittier (Manual de las Plantas Usuales de Venezuela, 110, 418. 1926), but unaccompanied by the name-bringing synonym necessary for the proper formal transfer of the name.

## Relation of temperature to the physiological values of salt solutions as indicated by growth of wheat roots<sup>1</sup>

SAM F. TRELEASE AND HELEN M. TRELEASE

The results of a previous study have shown that the rate of root growth in very young seedlings may be markedly influenced by the molecular proportions of  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$  contained in the culture solution supplied to the roots (Trelease and Trelease, 1925). Since these experiments were all performed with a temperature of about  $19^\circ \text{C}$ ., it seemed important to test other temperatures, to determine whether temperature might have a pronounced influence upon the relative physiological values of such a series of culture solutions. This seemed of special interest because experiments with longer culture periods and for later developmental stages of wheat plants had shown that the relative physiological values of a number of markedly different culture solutions may be either the same or more or less different, according to the nature and magnitudes of climatic differences (Trelease and Livingston, 1924). The present paper gives the results of preliminary tests of the relative physiological values of a series of solutions at  $14^\circ \text{C}$ .,  $19^\circ \text{C}$ ., and  $30^\circ \text{C}$ .

### METHODS AND RESULTS

The culture methods were essentially the same as described in previous papers (Trelease and Trelease, 1925, 1926). Seeds of a pure-line spring wheat (Marquis, Saskatchewan, no. 70, supplied by the University of Saskatchewan, through the kindness of Professor Manley Champlin) were soaked for three hours in tap water and then sprouted on wet filter paper in moist chambers. Each culture vessel consisted of a Pyrex beaker (tall form, without spout, 300 cc. capacity) having a piece of paraffined bobbinet stretched over the top and fastened by a ligature of paraffined linen thread. The beaker was placed in a similar beaker of 600 cc. capacity, and both the inner beaker and the space around it were filled with solution, the level of the latter being even with the top of the smaller beaker.

<sup>1</sup> Contributions from the Department of Botany of Columbia University, no. 348.

When the primary root of each seedling was about 6 mm. long, the seedlings were placed upon the netting at the surface of the solution, so that every root dipped into the solution while the seed was exposed to the air. Duplicate cultures, each of 25 seedlings, were grown. The cultures were kept in darkness, in a culture chamber with thermostatic control (Trelease, 1925). During the first two days each culture was covered with a watch glass.

In measuring growth rates two methods have been employed by various investigators. The first involves a comparison between the time periods required for various sets of plants to make equal amounts of growth, while the second uses a comparison between the amounts of growth made in equal time periods. Of course, both sorts of comparisons may be made if growth measurements are made frequently, at relatively short time intervals. Although the first method has theoretical advantages (Osterhout, 1922), its use involves great experimental difficulties. The second method was therefore used in this preliminary study.

In studying growth at different temperatures the duration of the test may be the same for the various temperatures, or it may be varied, according to the temperature. The latter procedure was adopted in the present work, since it may be expected to bring the plants at various temperatures more nearly to the same physiological stage of development. For each temperature the duration of the test was the time required for the roots in a standard or control solution to elongate about 84 mm. (from 6 mm. to 90). The time thus required was about 80 hours at 30° C., about 102 hours at 19° C., and about 173 hours at 14° C.<sup>2</sup> The standard solution (number 6 in table 1) contained 0.02 M  $\text{KH}_2\text{PO}_4$ , 0.02 M  $\text{Ca}(\text{NO}_3)_2$ , and 0.02 M  $\text{MgSO}_4$ . At the end of the test the length of the longest root of each seedling was recorded. For each culture, the mean initial root length (about 6 mm.) was subtracted from the mean final root length, and the value for elongation thus obtained was

<sup>2</sup> For this solution, the temperature coefficient,  $Q_{10}$ , for the range 14°–19° C. is 2.87; for the range 19°–30° C. it is 1.25. (See: Kanitz, A. *Temperatur und Lebensvorgänge*. 175 p., 11 fig. 1915.) The temperature characteristic,  $\mu$ , for the range 14°–19° C. is 17,600; for the range 19°–30° C. it is 4,060. (See: Crozier, W. J. On curves of growth, especially in relation to temperature. *Jour. Gen. Physiol.* 10: 53–73. 1926.) The values of these indices of course differ for different portions of the temperature range, and for different culture solutions, etc.



expressed as a percentage of the average elongation for the standard solution. Thus, each value for elongation represents an average growth rate during a time-period defined by the average growth rate in a standard solution. The results of the various experiments are summarized in table 1. Each value for an individual test is the average of two duplicate cultures of 25 seedlings each, except for the second values in the 19° C. series; the latter are derived from earlier experiments (Trelease and Trelease, 1925), in which the temperature, though not artificially controlled, remained close to 19° C.

When the variations among the results of individual tests are taken into account, it is apparent that the temperature of 19° C. did not differ sufficiently from a temperature of 14° C. to produce very important corresponding differences in the way in which the plants reacted to the various culture solutions. The average values for each solution are nearly the same; with these two temperatures the greatest difference for any solution is 7 (about 15 per cent of the lower value), but the evidence is not strong that even this difference is significant. On the whole, the results indicate that the temperature difference between these series was not sufficient to bring about any very great differences in the relative physiological values of these solutions.

By comparing the results of tests at 30° C. with either those of tests at 14° C. or at 19° C., it will be seen that, although the values are nearly the same for seven of the solutions, a pronounced difference is evident for solution 10, and less marked but probably significant differences are also indicated for solutions 7 and 9. These three solutions agree in having relatively high ratios of  $\text{KH}_2\text{PO}_4$  to  $\text{Ca}(\text{NO}_3)_2$ , but the series of data secured is not sufficiently large to warrant a discussion of the fundamental chemical or physiological conditions that determine the character of the results. Aside from these three cases, minor differences that may have significance are those between the physiological values of solutions 1 and 3 for 30° C. and 14° C.; the value indicated for each of these solutions is considerably higher for the 30° C. series than for the 14° C. series. A larger number of tests would undoubtedly reduce the "experimental error" and make possible more precise statements regarding the relations indicated by these preliminary experiments.

The results of these preliminary tests indicate, as would be

TABLE I

*Salt proportions of culture solutions, having total volume-molecular concentration of 0.06 M, and corresponding relative amounts of growth made by roots of wheat seedlings at 14° C., 19° C., and 30° C.*

SOLUTION NUMBER	MOLECULAR PROPORTIONS OF SALTS			TESTS AT 14° C.		TESTS AT 19° C.		TESTS AT 30° C.	
	KH <sub>2</sub> PO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	MgSO <sub>4</sub>	Individual tests	Ave.	Individual tests	Ave.	Individual tests	Ave.
1	5.0	5.0	90.0	53, 52, 54	53	61, 57	59	64, 70, 61	65
2	5.0	47.5	47.5	84, 93, 87	88	87, 84	86	83, 86, 91	87
3	5.0	90.0	5.0	55, 66, 61	61	62, 74	68	75, 76, 76	76
4	15.0	15.0	70.0	87, 95, 92	91	89, 94	92	89, 93, 95	92
5	15.0	70.0	15.0	78, 88, 83	83	83, 91	87	88, 86, 85	86
6	33.3	33.3	33.3	100 (control)	100	100 (control)	100	100 (control)	100
7	47.5	5.0	47.5	99, 91, 86	92	95, 82	89	98, 99, 105	101
8	47.5	47.5	5.0	86, 98, 89	91	95, 90	93	86, 94, 95	92
9	70.0	15.0	15.0	94, 86, 77	86	92, 90	91	102, 101, 112	105
10	90.0	5.0	5.0	75, 57, 56	63	74, 66	70	99, 96, 103	99

expected from theoretical considerations, that the comparative physiological values of a series of different culture solutions for a given kind of plant depend upon the temperature at which the values are determined. An adequate study of the responses to culture solutions differing in chemical or osmotic properties should involve tests at a series of different temperatures, and, conversely, a study of temperature relations should include as complete as possible a series of different culture solutions. The composition of the optimum culture solution for one temperature may be different from that for another temperature, and the optimum temperature may differ according to the nature of the culture solution employed. It is evident that the relative influence of any given culture solution upon a given plant must be considered as determined by the general environmental complex of influential conditions (Trelease and Livingston, 1924), including chemical and osmotic properties of the solutions, temperature, vapor-pressure deficit and velocity of air movement around aerial parts of the plant, quality and intensity of illumination, etc.

#### SUMMARY

By comparing a number of culture solutions by tests at 14° C., 19° C., and 30° C., it was found that for different temperatures certain of the solutions had nearly the same relative physiological values (for root growth in very young wheat seedlings), while for different temperatures other solutions had markedly different relative physiological values.

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# INDEX TO AMERICAN BOTANICAL LITERATURE

1916-1926

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BULLETIN  
OF THE  
TORREY BOTANICAL CLUB  
DECEMBER 1926

The present crisis in plant physiology<sup>1</sup>

BURTON E. LIVINGSTON

The last decade has been characterized by a rapid and remarkable development of a world-wide consciousness of the rôle of science in human life. Although this consciousness has been long present in some minds and sporadically evident in the writings of some authors, it has only recently diffused and become well-nigh universal among thoughtful people. Practical application of scientific knowledge in the ordinary affairs of life is now generally and widely appreciated and the scientific method of thought is everywhere in demand for the production of physical necessities, conveniences and luxuries. Everyone knows that science is in the midst of remaking the physical and biological world. There is strong demand that it be especially prompt about all the details that so obviously have needed intelligent attention for so long.

Also the ethical and aesthetic values of scientific accomplishment and scientific culture are being increasingly appreciated in this period of regeneration, whether with brave joy or with fearful misgiving. Many careful thinkers are able to see in the rapidly increasing body of scientific knowledge the promise of an approaching release from many of the spiritual as well as material troubles of mankind; *veritas scientiae vos liberabit*, for science is remaking the spiritual world as well as the more tangible and directly familiar one. The conscious purpose of far-reaching human intelligence which really approaches omniscience from the standpoint of primitive culture, although now

<sup>1</sup> Based on an invitation address presented before the joint meeting of Section G (Botany) of the American Association for the Advancement of Science, the Botanical Society of America, the American Phytopathological Society, and the American Society of Plant Physiologists at Kansas City, Mo., 29 December, 1925.  
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only in its own embryonic phase, is bent on improving the world for human needs and sensitivities, by sorting and arranging an apparent chaos of seemingly random happenings. In this particular period of heightened intellectual activity and spiritual yearning, the beginnings of a race consciousness seem more evident than ever before in similar periods. It may be that the universe conscious of itself—long dreamed of or mystically hypothesized—may be developing at length, at least in the form of the human world conscious of its past and present and bold to take charge of its own future at whatever risk. Weak as it still is, this embryonic race consciousness is already highly significant. It is armed with the recently developed methods of science and it aims to move steadily forward in the understanding and appreciation of humanity and human surroundings. Imagined by many cults as already existent somewhere in the voids of the universe, or perhaps permeating it like an imponderable ether, this driving purpose toward natural betterment, toward good things that are to be secured for humanity, here on our planet, seems now to be actually recognized, evolving and emerging in our very selves. Is the mythical *Zeitgeist* really turning the future of humanity over to this human spirit of conscious progress?

On the other hand, many people in their outlook on the unknown are guided by a familiar conservatism of feeling, refusing to risk the flights of thought excepting in practical affairs. These seem generally not yet to have grasped the deeper implications of physical and biological accomplishments. They protest strongly against scientific progress as a terrible disease of the ancient system of spiritual values, which must somehow be checked in its ravages—even by blood-letting and the heroic infliction of pain (on others), if such things should prove necessary. No one of any class, however, appears to oppose science as seriously undesirable from the standpoint of ordinary practical affairs, and its advance can now derive the very salutary stimulation of opposition only from reactionary views on moral, ethical and aesthetic values. Indeed, those who lead in decrying the philosophy of science apparently find no great difficulty in accepting with free acclaim the quotidian conveniences and wealth-producing opportunities with which scientific endeavor has already variously flooded the world, and a few of these

clamorous opponents of the freedom of thought are actually engaged in scientific research in some field where results may be applied and perhaps lucrative, without too seriously disturbing the moral and religious feelings of any one. Science aims toward consistency and its opponents should of course be inconsistent with respect to its values.

So our stage is set for the entrance of scientific humanism and we live in the earlier years of what promises to be a new renaissance period. Science is encouraged and supported as never before, and there is enough of prejudiced opposition to arouse us to our best efforts for an ever increasing and broadening appreciation of ourselves and of the world about us. The atmosphere is tense with new knowledge and new inductions, which will soon strike here or there. The world is on its toes to see each new flash. In all their various fields the followers of each science are thoroughly aroused. The progress of discovery and interpretation is greatly accelerated, with an acceleration that is itself continuously accelerated. The present represents a crisis in every branch of science, and we may well examine our several immediate fields of work at this time, to appraise, as best we can, what has been done and to plan for the future with as much broad sanity as we can muster.

In all the fields of science there has developed or is rapidly developing a productive interest in the deeper aspects of the things dealt with. Much has been accomplished in the primary discovery and simple description of forms and colors and other characteristics or properties of objects, much has been done to give us definite knowledge of the arrangement of things in space and of their sequence or succession in time, and now the dynamic aspect of phenomena, with special reference to changes and their causal relations, is becoming increasingly prominent in scientific discussion. The new period into which we are entering, if one may judge so early, appears to be peculiarly characterized by this dynamic point of view. We are in general no longer content with the facts of simple observation, we desire to understand how and why things have come to be as we have found them and how and why they change from period to period and differ from place to place. Perhaps this recent trend of thought toward the more frequent consideration of causal relations in a quantitative way, especially in the

physical and biological sciences, may be regarded as the basis for the rapid rapprochement of science and philosophy that is apparent on every hand.

One of the most remarkable and apparently healthful of recent developments in intellectual affairs is an increasing tendency to disregard or even ignore the older, established but superficial lines of demarcation by which the several fields of knowledge have been so nicely separated. The insulating partitions are disintegrating and thought may now range with relative freedom throughout the entire universe. Workers in one established and named branch make expeditions into other fields and bring back great wealth of new ideas; sometimes they do not return at all but remain at a boundary and enrich two or more branches by their work. Newly developed sciences in many cases occupy border regions and appropriate terrain from several of the older disciplines.

The classification of knowledge is thus being remade. Note how physical science has permeated biological science, how biological studies become starting points for far-reaching progress in the physical sciences, how biology is bringing its parts together into a coördinated whole, how the physiology of plants and lower animals and human beings is being welded into general physiology, how physics and chemistry and astronomy and geology are blending and amalgamating in the hot flux of recent thought at many of their points of contact. Note how philosophy becomes more scientific and science more philosophical, how cosmology and eschatology and even ethics are progressively influenced by natural science, as the findings and methods of the latter slowly permeate into those ancient realms of the free imagination. Note, also, more tangible things: how two or more sections of this American Association combine recently, to hold joint sessions for border-line discussions, how sessions for the discussion of genetics are planned for this aspect of zoology and botany together, how many books appear on physical and mathematical science for biologists—though I have not yet observed so many on biological science expressly prepared for physicists, chemists and mathematicians. Perhaps the next form of classification of the intellectual fields may be largely based on methods of thought and experimentation rather than on superficial characteristics of the things studied. We

are beginning to try cutting our loaf in new directions and on un wonted lines.

The new era is also notably characterized by interest in science as a whole and in the scientific method in general. The emphasis now being placed on the interrelations between the various branches of knowledge leads to this broader interest and to it is added an importunate demand for the use of the scientific method in all fields of human activity. Industry and art call for scientific research at every turn and the intelligent public desires to know what is going on in research and what may be the general meaning as well as the practical application of each new finding. In every civilized land alert youth, together with many of its elders, is critically examining the organized educational systems of past and present. The resulting conscious desire to improve education is leading to greater emphasis on science in general, less on the particular sciences. The recent rapid growth of the American Association is one sign of the active response of scientific specialists themselves to this broadening influence, for the Association stands preëminently for science as a whole. The rapid development and remarkable success of the National Research Council furnishes another evidence of this remarkable awakening of consciousness among men and women of science in the United States. Not only the public and the press that tries to inform and guide it, but also the research workers themselves, are vitally interested in movements toward the reorganization of the science personnel and the science field. The hope is everywhere expressed that science may eventually organize itself as satisfactorily as it seems already to have organized many fields of art and industry, and that it may consequently furnish humanity with better facilities and greater opportunities for securing the higher pleasures as well as those of more primitive kinds.

#### GENERAL ORGANIZATION OF PLANT PHYSIOLOGY

Turning now to plant physiology in particular, if it is to maintain its present prominent place among the other sciences and to go forward with them, it must needs become more conscious of its means and aims than it has ever been. Closely related, both by its content and by its philosophical implications, to animal and human physiology and to psychology, as

well as to the physical sciences, plant physiology lies in the region where physical and biological thought meet and blend.

This dynamic branch of the *scientia amabilis* appears to require some reorganization and reclassification of its subject matter, critical examination and improvement of its experimental and logical methods, and improved arrangements to facilitate the dissemination of ideas and the ever more necessary interchange between it and the related sciences. Rapid progress is being made in these things. Especially do the new times call for a larger degree of individual coöperation than has characterized our field in the past and the organization of convenient, comfortable, profitable and efficient means by which many minds and hands may unite in efforts to solve the problems that lie next in our program, so many of which are now in a state that renders individual attack upon them well-nigh hopeless. As was said by the late President Bessey in his address before this Association at Cleveland, in December, 1912, the period characterized by guerrilla methods in the conquest of the unknown is drawing to a close, and suitable organization of research workers becomes increasingly essential.

A widespread desire for improvement in the organization of plant physiologists in this country has just given birth to a new society. Publication facilities for American plant physiology are being increased and promise to be more satisfactory than ever before. The series of monographs called *Physiological Researches*, inaugurated too soon, perhaps, was caught in the trap of financial circumstances and recently came to an end with the close of its second volume; but another journal, *Plant Physiology*, has just been launched under the auspices of the new society. Its pages are to be open to contributions from members and non-members alike and it aims to present only exceptionally high class work. The regularity of its publication (it has begun as a quarterly) will perhaps make this aim somewhat difficult to carry out, but we may be sure that, as far as the available papers will allow, high standards will be maintained. The quality level of its contents clearly will depend on the papers submitted for publication. The recent launching of the *Biological Review* is an important movement for plant physiology as well as for biology as a whole. The inauguration of *Protoplasma* is of specially great importance in the field of

protoplasmic physiology. And physiologists are appreciative of the excellent facilities offered by the recently reorganized *Journal of Agricultural Research* and by *Soil Science*, which likewise has completed a series of volumes and is continuing, as are also the older journals and other serials that present physiological material.

Most important has been the development of *Botanical Abstracts*, the finest project thus far undertaken by American botanists, a project unthought of ten years ago. In some respects it is patterned after *Chemical Abstracts*, which is considerably older. The new renaissance movement in science is well illustrated by the remarkable success of these and other abstract journals. *Botanical Abstracts* is soon to be combined with others in the biological field and enlarged to form *Biological Abstracts*, which is a still greater and broader undertaking, by all sorts of biologists under the auspices of the Union of American Biological Societies. The Union is another very important development of the last few years, illustrating the recent tendency toward active coöperation among the numerous and varied branches of biological science. On the whole, publication of research in plant physiology is being fairly well cared for, the abstracting and indexing of current work is being excellently well done and the future is bright along both these lines.

As to its content and its problems, plant physiology is turning, with the other sciences, to the consideration of questions of dynamics and quantitative causal relations. It is emphasizing the quantitative aspect of its observations and is rapidly moving toward really controlled experimentation for the solution of questions of causality and deterministic control. Hand in hand with physics and chemistry and the rest of physiology, it has so rapidly advanced during the last twenty years that Kostychev can say of its chemical aspect, that only the framework of the subject has survived these two decades of advance and change. This statement is just as applicable to the physical aspect and to the field of irritability and response.

In the following pages of this paper I wish to consider several critical aspects of our science, with reference to its recent past and its probable future. My topics are: Presentation of results of research, The summarizing of research and the providing of text-books, The general exchange of ideas among investigators,

and Research problems, methods and facilities. To the last topic I shall devote most attention.

#### PRESENTATION OF RESULTS OF RESEARCH

Facilities for the publication of the results of original research in plant physiology are, as I have said, now fairly satisfactory. There is, indeed, no present reason for misgiving in this connection. Research workers are now being asked to write articles for journals and for other channels of publication, and some editors are, I have heard, sometimes a bit put to it to fill their pages. I have myself recently received requests for papers from no less than four scientific journals. It is true that, since most journals require variety in their output and are limited as to size, their editors have to refuse papers that are too long; but short, original articles carry farther and are read more quickly and generally do more for a science, page for page, than do longer ones. Nor is physiology apt to be seriously retarded because of lack of funds for the publication of costly illustrations. Our science has no great need for plates and expensive figures; in general, we do not need to prepare them, we do not need to find funds for their reproduction, and we do not need to handle them in our reading. For this, our field is now much like that of physics and chemistry, but not all scientific branches are so fortunate in this respect. We do, however, need to publish many tabulations and graphs.

It is an interesting observation that recent discussions of the need for improved facilities for research publication have generally contemplated the problem from the standpoint of the author rather than from that of the reader. It has sometimes seemed as if the scientific journals exist and operate primarily to publish John's paper, or to advertise John's institution, with only secondary thought to supplying Richard with helpful and valuable information and new ideas. Doubtless John, as an author—perhaps pressed by his institution—is more importunate and influential than is Richard, as a reader, and you may think of several reasons why this is so. It seems, however, that there is a growing tendency for Richard to clamor a bit now and then for better scientific reading or reading more nearly suited to his wants, and the editors of our publications are not deaf to Richard's wishes, especially since he often appeals to



them in financial terms. I think American scientific editors are coming to exercise their right of choice and criticism more than previously, and I regard this as an excellent and upward-pointing sign.

Not only readers and editors, but also authors, will need to take still more thought in this matter of the quality of research publications, if plant physiology is to proceed as we think it should. We need to realize that, while it does take much time and energy to carry out a piece of research and prepare an article about it, it really consumes, in the aggregate, much more time and energy to read the published account, if a large number of people indulge. The readers should surely be considered, not only those who are with us but also those who are yet unborn. I wish here to refer to the form of presentation only, assuming for the moment that the material to be set forth is unquestionably worthy of presentation.

If you will look critically into any dozen recently published papers in our science, I think you will agree with me that American plant physiology is in grave danger, unless marked improvement can somehow be brought about. We are all too generally hasty and even careless about clearness as well as about succinctness. We often fail to say just what we mean and we not infrequently say things that we do not mean. Our logic is often greatly at fault, our vocabulary and our facility with words are apt to be far too limited. We seem to have come upon a time when a degree of lack in thought precision and in a sense of neatness in literary composition causes less embarrassment than does a corresponding degree of lack in personal dress or table manners. The research worker in science and especially in physiology, now holds the key to the romance of the newer time, and it requires a high degree of art to transfer the products of his thought and scientific imagination from his own mind to the minds of others.

Plant physiology is fundamental to a far-flung galaxy of applied sciences, upon which, as is so often and tritely remarked, largely depend the feeding and clothing and housing of humanity as it increases and fills the world. Consequently, many who are not specialists in our field need to read our research contributions. This is one great practical reason for giving necessary attention to the literary side of our work. We supply

our products to many kinds of minds and if we are fair salesmen we shall try to market these products in convenient and efficient, even attractive, packages. Furthermore, science is international and polyglot; perhaps more of the readers of your research article will know English only as a book language than will have it as their mother tongue. For that large—or at least more widely scattered—group of readers in the present and in the future, clearness and precision of statement are specially requisite. Finally, the recent recruits to any science, from whose number will necessarily come the leaders of the next generation, must begin to read and use our original contributions long before they can have cultivated a knowledge and enthusiasm that will make up for our carelessness or superciliousness or plain laziness in literary composition.

From these different points of view, and from many others also, our greatest present need is not for more or more prompt publication of research in our field, but for better presentation on the part of our authors themselves, who may well bear in mind—what may sometimes be a bit startling—that the main reason for scientific publication is, after all, to inform and stimulate and please the readers. It will not do now for us to hide our literary weaknesses behind the thought that a reader is proved to be stupid if he is unable to get out of our papers what we vaguely intended to put into them. It is clearly the author's business to guard against possible stupidity or ignorance on the part of his readers. Writing can generally be done in periods when we are at our best mentally and when time may be taken to look up questionable points so as to care for many aspects of our presentation. On the other hand, reading is generally done when the reader's mind is more or less passive; a reader likes to be carried along by the author, without much necessity for constructive thinking on his own part and without having to make up for hiatuses or other imperfections in the author's composition.

One of the most frequent inadvertences of recent American writers in our field is failure to provide the reader with adequate orientation and with lucid interpretation. A few sentences of orientation help very greatly to catch the interest and stimulate the imagination, and most readers need that sort of help in most cases. Many who should be greatly interested in our

reports will surely fail to realize that fact unless we provide an intellectual ramp in each case, upon which their minds may easily come to the level of our thought. These are not stupid, they are merely engrossed with their own work on their own levels. Good ramps—sometimes even elevators—are needed.

Similarly, it is not to be expected that many of those who have use for our contribution will take pencil and paper and work over our published results to extract the meaning they bear. Great care is generally required to make sure that our statements may be both clear and as brief as is consistent with clearness and easy reading. Like a beginning student in an elementary laboratory course, the average reader needs to have his attention called to the things he is to see. He may subsequently either agree or dissent, but he has a right to expect us to show him clearly what are our conclusions and how we come to them.

One more point and I shall have done with this sort of preaching. Graphs and diagrams and sometimes pictures are, as every one knows, an enormous help in the transfer of ideas from one mind to another. These are frequently essential in plant-physiological presentations. But they should be illustrations, throwing light on the text; they cannot take its place. Different minds note different things in the same drawing, and actual statements are needed whether illustrations are employed or not. I think most readers of our contributions will be appreciative and thankful if we make definite statements in words of whatever we think should be remembered, even when these points are shown by our illustrations. A science such as ours must have clear expression in sentences; it cannot dare to risk possible miscarriage of ideas such as is apt to occur from the cursory examination of published figures.

#### THE SUMMARIZING OF RESEARCH AND THE PROVIDING OF TEXT BOOKS

We all agree that the abstracting and indexing of our current plant physiological literature, as of current biological literature in general, is in good hands and is going forward satisfactorily. Conditions in this regard have completely changed since 1918. The machinery of *Botanical Abstracts*, and now of *Biological Abstracts*, seems to be adequate for the near future, and we may feel sure that improvement will be continually made. With

regard to summaries and hand-books of plant physiology, we are not nearly so well cared for, though some excellent monographs on special parts of this field have recently appeared. The literature is now far too extensive and various to be grasped by a single mind, even with the great aid of abstracts. A comprehensive hand-book as satisfactory as Pfeffer's was for its own time is not apt to be produced ever again. Future summaries will almost certainly be monographs on separate phases and by different authors or different groups.

Perhaps the greatest literary need of our science at present is for comprehensive monographs of this sort, such as Stiles's recent one on photosynthesis or Burgerstein's on transpiration. This great need should be consciously cared for in some regular and consistent manner; the preparation of monographs ought not to be left entirely to individual initiative. Perhaps the technical societies might make this a regular part of their service to their members and to the science, arranging for the preparation and publication of monographs and for their revision from time to time. The main difficulty is not the printing and distribution but the securing of suitable manuscripts; that is, finding authors and editors who can and will do the work, which must be largely labor of love.

We now experience great need also for advanced text-books and for elementary treatises. The difficulties before us here are similar to those just mentioned. Perhaps the advanced texts of the future, and possibly even the elementary ones, may be of coöperative authorship. We may derive suggestions from other branches of science, but these general projects will need to receive serious attention from the ablest plant physiologists.

In connection with the suggestion that arrangements for monographs might be taken up by organizations devoted to plant physiology—and arrangements for text-books might also be cared for by such organizations—it may be possible for a scientific society actually to publish such books itself (just as societies publish journals), or for a group of societies to form a publishing association for that purpose. Such a plan, if it might be successfully worked out, should greatly benefit the science. If we consciously and seriously consider all ways and means for advancing our science, some such suggestion will be worthy of thorough study.

## THE GENERAL EXCHANGE OF IDEAS AMONG INVESTIGATORS

The complexity of the problems in a field of plant physiology makes it highly desirable that investigators in such a field should have easy and natural means for the exchange of ideas in ways less formal and stereotyped than by the printed page. This thought has been back of the organization of scientific societies, and the annual and other meetings of our societies are of very great value in promoting natural personal intercourse among those who attend. But the programs of these meetings are now generally so filled with papers formally presented that they do not sufficiently encourage and facilitate the sort of intercourse and mutual criticism that all desire. Much constructive thought needs to be given to this phase of our work and it is beginning to receive attention. I meet everywhere with the suggestion that a program committee might place on its program only a relatively few selected papers to be read, leaving much time for discussion, but this suggestion is not often carried out as yet. It will not be sufficient simply to provide time for discussion and criticism. These need to be encouraged by preliminary arrangements that are often more important than the selecting of formal papers. It might be well to give regularly an hour or more to a properly selected paper, rigidly confining the author's reading of the paper itself to a small fraction of that time and having two or more other persons prepared and ready to criticise and discuss the presentation. If discussion were thus started it would generally continue, I think, being taken up by others of those present.

The presentation of five or six ten-minute contributions in an hour at annual meetings of our large societies will need to be discontinued if our meetings are to be as useful as they should be. These meetings should proceed in a leisurely manner with much time purposely left unoccupied by the program itself, time that may be used for personal and informal conferences. Afternoons generally might be left open in that way. The purpose of a meeting might be to promote and facilitate informal discussion and the exchange of ideas, a few properly selected papers being placed on the program to stimulate this exchange. Another promising suggestion is that there might be an exhibition in connection with each meeting and that certain periods be set aside for visiting it and conferring with the exhibitors,

who would be present for that purpose. A striking demonstration is a great catalyzer of discussion and thought.

#### RESEARCH PROBLEMS, METHODS AND FACILITIES

Interest in plant processes probably first arose from a desire to understand how plants develop, how they grow larger, mature, *et cetera*. These processes or changes are now the subject of our special science. As different sorts of physiological change were discovered and described, the problem of process rates became more prominent and people wished to know when the life changes were slow, and when they were fast, and when they ceased altogether. With some of these questions about time relations and simple sequence more or less satisfactorily answered attention began to turn to the more detailed *manner* in which the rates of plant processes change from time to time, and finally physiology began to come fully to its own with the emergence of problems of causation. At present we are largely occupied in trying to find out the antecedent conditions that determine the processes that go on in the plant. It is specially in connection with these newer problems of causal relations that we now find ourselves in a critical position. For these problems qualitative observations are of but little value, and emphasis is now being strongly placed on quantitative experimentation.

The last decade or so has been specially characterized by this turning from predominantly qualitative to increasingly quantitative work in plant physiology. The standard plants (commonly called controls or checks) of our experiments are given greater prominence and they are selected with ever greater care and receive ever more attention, since they form the bases or datum points from which comparisons are to be made. On the other hand, for the variously treated plants or tissues or organs in an experiment the particular treatments are now generally more definitely known than heretofore. It has been found that we cannot hope to fix upon the exact influences that control a plant process unless we have very precise quantitative knowledge of all the influential conditions, as well as of the process itself, as its rate differs with different treatments. Whether the externally influential conditions of any experiment are artificially made and controlled or are naturally produced, it is now realized as essential that all of them should be quantita-

tively described, precisely enough to render the entire environmental complex reproducible, or at least capable of being identified if it is ever encountered again. Unless the conditions of an experiment are known in such a way, the experiment is of little or no value, and inferences of causal relations cannot be satisfactorily drawn. It is therefore absolutely necessary that we have available apparatus and methods by which data may be secured that will adequately describe both internal and external sets of influential conditions.

Our ideas as to what should constitute good or even passable experimentation with plants have been almost completely changed in the last quarter-century. It used to be taught that a perfect experiment consisted in maintaining all but one influential condition and letting the single remaining condition differ from test to test in known ways and to known degrees. But if we go no further than that our results will mean very little and we shall have nearly wasted our time, as far as really advancing science is concerned. It is essential that the maintained influential conditions be just as precisely known and described as is the variable one. Otherwise we have no logical basis for any definite conclusions and our experiment cannot be repeated, or even recognized if it should ever happen to be repeated. Most physiological experimentation is very poor indeed, excepting as preparation for the use of better methods, unless this very fundamental and very obvious consideration is held in mind.

We have now arrived at a stage in the progress of our science where the things just mentioned are becoming generally appreciated by research workers. But the apparatus and methods by which effective conditions may be controlled and measured are generally not yet available; they remain mostly to be devised. The present crisis finds us fairly well able to recognize the essentials of valid experimentation and reasoning, but we are generally unable as yet to carry out experiments that will fulfill our specifications. From this sort of impasse there is only one rational escape. We cannot now go back and again content ourselves with simple sequential descriptions of what we observe to happen in our plants, nor can we go on indefinitely guessing at causal relations without the quantitative data that are requisite for logical analyses. Opinions as to possible causal

relations are now directly interesting in our science only if they lead to improvements in experimentation and methods of reasoning. The only escape from the impasse lies in devising and employing adequate instruments to give the necessary numerical data and in devising and employing adequate methods for treating such data in ways to bring out any causal relations that may be uncovered by our experimental procedure.

It is almost as though a new world had been opened for exploration. Our whole field lies before us in the new light, all unexamined with respect to quantitative causality or deterministic control. You can scarcely make a single statement about plant processes without raising new and enticing problems that seem to be approachable by experimental methods that we should now be able to work out. Contemplated in this way, these are indeed thrilling times. But our next expeditions into the unknown need to be manned and outfitted with a care and thoroughness that were never required before. We have mental habits that must be watched carefully or we shall be likely to go forward along circular paths. This change in our science must come gradually, for the older fashions of the great pioneers are still prevalent and will remain so; there will perhaps never be a time when qualitative experimentation and opinionated discussion will not occupy prominent places.

During the period when physiology has been turning to quantitative methods and more rigid analysis for discovering causal relations, its content and thought have been adopted almost wholly by pathology and ecology and all that group of applied sciences which comprise the applicational aspect of ecology. Much of pathology and a good part of ecology have become physiological and the same is true of agronomy, horticulture and forestry, which are the ecology of cultivated or practically useful plants. These now deal largely with physiological problems. Physiology as such would probably have come eventually, but perhaps more slowly, to the quantitative study of causal relations; but its natural tendency in that direction has been greatly hastened by the demands of pathology and the various branches of ecological study. The applied sciences require just as much knowledge of determining conditions as can possibly be secured, for their main aim is to develop the arts of plant production and conservation by informing



growers and handlers of plants how to control plant growth, etc., by the modification of natural conditions or the substitution of artificial ones. Only through knowledge of causal relations can we hope to accomplish these ends. So plant physiology is driven forward, by practical demand as well as by the logic of its own problems, to questions of quantitative dynamics and of the deterministic control of the vital processes.

On the other hand, the superficial and generally hurriedly planned physiological experimentation of ecology and the related applied sciences tend somewhat to retard progress toward more refined types of study, because their problems are so numerous and insistent and because many of them are commercially and politically and economically so important. These branches demand experimental results promptly and in large number and their workers generally have little time or energy or patience for working out the most satisfactory kinds of experimentation and interpretation. With some pronounced exceptions, their experiments are not generally satisfactory to thorough-going physiology, excepting in a suggestive way, but they do serve to maintain interest and they sometimes indicate empirical solutions of practical problems in plant growing. Perhaps they retard physiological progress somewhat by their common tendency to disregard the logic of careful analysis, frequently attempting to substitute short-cuts in place of well planned and thoroughly interpreted experimentation. Some may be led to regard these attempts, which are commonly just empirical tests made for practical purposes, as truly physiological experiments.

But the deeper physiology has gained much from this kind of work, superficial as it confessedly is, and it is well to remember that our science has reached its present state largely through such studies. We should not now be about ready for deeper-going and more thorough investigations if so much relatively superficial and empirical work had not been done. Inadequately planned and carried out, partly observational and partly experimental, with only a few of the essential variables quantitatively measured, and with inadequate experimental control and only superficial interpretive analysis, such pioneer work served our science through its earlier phases of growth and must continue to be very useful, especially in general education and in the applications. But the best physiological work can no longer be after that pattern.

As I have said, experimentation on the relations between the rate of a process and the conditions that determine that rate essentially requires that numerical indices be secured to represent the process rate itself and also the intensities or rates of all the influential conditions; but we can seldom fulfill these requirements to a satisfactory degree. We are generally still unable even to enumerate all the conditions that should be measured or controlled.

Preliminary experiments are generally necessary to find out what are the main influences that need to be considered, and the best present work is largely in such preliminary experimentation. Even for the influential conditions with which we are qualitatively acquainted, we are generally not yet able to secure numerical indices, either for want of proper instruments and methods or for want of knowledge as to how to handle and apply quantitative numerical data after they have been secured. It is thus seen that, although we are bent on the quantitative study of causal relations, we find it thus far impossible to carry out experiments according to our ideals or in ways that even approach our ideals. I judge that hardly a single passingly satisfactory experiment has ever been performed with plants. The group of unknown or unmeasured influences called "chance" have played too great a rôle. As Francis Bacon knew, chance needs to be eliminated from science generally and this is especially true in respect to physiology.

This condition of affairs is characteristic of the present crisis. We begin to know what we wish to do and we enthusiastically attempt to do it by inadequate plans and procedures, although we are aware beforehand that our experiments will generally fail. We secure results that may be quite adequate in some respects but just as hopelessly inadequate in others, thus defeating our own purpose. We publish "little contributions with blunted conclusions," as far as causal relations are concerned. We spend our money in printing elaborate tables of numerical data that represent the precise truth for a certain set of unmeasured conditions, which, however, we do not and cannot define. Our most careful measurements and integrations are too frequently expressed in terms that do not properly apply to the problem in hand and we know not how to weight or interpret them so as to make them satisfactorily applicable.

It is no wonder that many of our experiments cannot be repeated and that our discussions teem with attempts to employ merely qualitative knowledge for the testing of quantitative hypotheses, or hypotheses that should be quantitative if they were really and permanently useful.

Such thoughts as these seem to be a bit discouraging in the first instance, for they indicate that we are undertaking to begin to read the book of physiological causation without adequate knowledge of its language, or even of its alphabet. We understand many letters and a good many words of that book, but most of its sentences and every one of its chapters confront us with relations for which we do not have the proper keys. But we must remember that causal relations in vital phenomena constitute the most difficult and intrinsically complicated subject with which human understanding has to grapple. Our problems are necessarily vastly more complicated than those of the physical sciences, for vitality is not a part of their field. Perhaps the only group of vital phenomena that are still more complicated than those of plants are those of nerve phenomena in the higher animals, including the field of psychology. Still, the living plant presents problems whose solution, it seems, would form a generalized basis for the study of nerve.

Of course it is not to be expected that the problems of plant physiology may lend themselves to relatively easy solution. Their intrinsic complexity is our only possible reason for discouragement and, looked at rightly, this very complexity constitutes the stimulation and challenge of our science. We shall not be discouraged nor pessimistic. *Ignoramus* is an important word in our present vocabulary, and it is a great achievement to recognize when and where we do not know, but the word *ignorabimus* is not ours. In the present crisis we may say, "We do not yet know but we intend to investigate and find out." We surely have no historical basis for thinking that lack of present understanding of complex phenomena is any reason for admitting the possibility that we may not be able to gain understanding by scientific investigation. Science has plenty of time to approach its goal and our only fear may be that we may be less efficient than we might be, thus failing to receive the highest pleasures of the finest intellectual art.

Problems involving the thorough treatment of determining

conditions will generally not be approachable by single investigators working alone, even after the needed methods and instrumentation shall have been worked out. For every physiological process there are a number of influential conditions to be taken into account and these generally fluctuate from period to period; consequently a useful set of quantitative data, for the study of any process in relation to the conditions that may influence it, can be secured only through the making of many simultaneous measurements at intervals throughout a long period. Even when we employ the few recording or integrating instruments that are thus far available for our work, the actual labor of conducting a well planned experiment on the causal relations of processes in living plants is generally much too great for any individual, no matter how active and dexterous and foresighted he may be. Also, many experiments require hourly or more frequent observations of plants and instruments throughout a period of twenty-four hours or more, and an investigator finds it difficult to maintain his best mental condition through such long periods. Finally, the artificial control of conditions like temperature and air humidity require rather elaborate instrumentation and attention from time to time. Whether we arrange an experiment with artificially controlled conditions or set about it to measure natural conditions, or if we attempt both together, we find that the outstanding problems now confronting us are all too complicated to be handled by any individual. Much can and will still be accomplished by individual workers, but it is practically certain that the great future experiments, by which adequate consideration of deterministic control will be brought eventually into our science, will be carried out more and more by groups of coöperating workers.

Here we have another characteristic by which the future of physiology promises to differ from its past. It has been said frequently that coöperation among scientific investigators is highly desirable, but for our fields it is now absolutely essential. The new period will be especially one of group operations. It will be necessary, as is already becoming evident in places where these broader and more exacting problems are taken up consciously and seriously, for a number of workers to pool their intellectual and technical ability and work together on the same general investigation. We can learn much from the

organized methods of industrial research and from the work carried out by some government and institutional laboratories. Ways must be found by which human individualism may be satisfied or sublimated without interfering with the consciously planned progress of great investigations. I have not the temerity to attempt to predict how this will be accomplished, but I do wish to emphasize the thought that such accomplishment is absolutely necessary.

When a group of workers, including several scientific investigators and their assistants, purchasing agents, mechanics, mechanics, computers, clerks, typists, laborers and janitors, shall combine their efforts to begin really planned experimentation on the control of plant processes, it will of course be necessary that they have special laboratory facilities, such as are thought of in connection with the best physical, chemical, meteorological and medical research institutes, but they will require much more. Excellent beginnings along some of the lines here suggested have been made in this country at the Boyce Thompson Institute for Plant Research and in some of the offices of the United States Department of Agriculture. It does not seem likely that suitable laboratories for such broad and complicated experimentation will be generally possible directly in universities, though the new type of research institute may well be connected in some highly satisfactory way with a new type of university that will probably soon begin to make its arrival evident. A point to be emphasized is that fundamental research will be the main aim of such establishments, the training of advanced students being no primary part of their activities. Nor will the insistent practical problems of agriculture or forestry be allowed to usurp the attention of the workers. There will always be, as there now are, many laboratories where attention will be primarily devoted to practical problems, but institutes for particular fields of plant physiology will need to devote themselves only to the fundamentals; they will need to have no direct connection either with experiment stations or with education as such. Of course they will derive ideas and probably workers from the fields of applied science and their assistants will naturally and automatically receive the best possible advanced training. These assistants will be employed to do needed work, much as in an industrial laboratory, and they

will learn and grow, but they should not generally receive fellowships nor should they work for degrees in the present mode. The details of housing and personnel organization will of course need to be worked out.

The realization of such a dream as this will necessitate large funds, for current expenses as well as for original equipment. Much of apparatus and supplies will need to be purchased and much will need to be devised and specially made. The purchase of ready-made instruments and other apparatus is of course a form of coöperation, the makers of the apparatus thus aiding the investigators in specific ways and receiving payment in return.

We may well cultivate familiarity with the general idea of group research in our science. The idea is already being put into practice little by little, in this branch as well as in others. It will surely come to be a dominant idea and a commonly accepted mode. We may well try to prepare the way for the undertaking, by groups, of such broad and complex and consciously planned research as I have suggested. What are the essential preliminaries in the accomplishment of which we, as individual men of science, may now engage?

As we have said, funds will be necessary. We may further this general idea, in our writing, teaching and conversation and in our interviews with news writers. Let it be understood that plant physiology is as worthy of and as ready for adequate research facilities and endowment as is astronomy, chemistry, general botany, medical science or any other field of scientific investigation. This field excels in opportunity for the advancement of pure science, by which I mean the philosophical appreciation of the universe; for plant physiology occupies the borderline between the physical sciences and the sciences that deal with human thought and feeling. The basic problems of protoplasmic properties and reactions may, in general, be best attacked in this field. We are probably not in danger of anti-vivisection laws, and experimentation with plants is probably not intrinsically repulsive to any one. This field also excels in possibilities for the advancement of the arts, for the application of scientific knowledge in agriculture, horticulture, forestry and the industries and vocations that deal with plant products. The relation of plant physiology to plant production and the handling of plant

products is similar to the relation of animal physiology to hygiene, medicine, and animal husbandry. The arts that rest on plant physiology are the most fundamental and necessary to civilization. Its applications in daily life are the practical business of the majority of human beings for most of their waking hours. Chemistry and physics have given to industry many processes by which plant products are modified, but these operations still depend upon plant growth for most of their materials. Whenever it shall come to pass that a good part of the organic materials for human food and protection may be synthetically prepared without resort to living plants, you may be sure that the keys to the new industries will come largely from plant physiology. Perhaps, as is often said, the most important process in nature, from the standpoint of humanity, is the photosynthesis of carbohydrate from water and carbon dioxide, and this process of green plants presents one of the basic problems of plant physiology as well as of chemistry. We are not apt to secure our carbohydrates without the use of plants until we shall have understood much better than we do now the physiological process or processes of photosynthesis. Indeed, the suggestion is patent that special institutes for the investigation of this particular field of plant physiology should be the first or among the first to be established.

It is remarkable that plant physiology has accomplished so much in its period of pioneering and individualistic researches, but the new times demand new and larger methods here as elsewhere, and laboratories for this science ought not much longer to be the poorly-equipped affairs with which we are all too familiar. If these thoughts and other similar ones may become familiar to the intelligent public there is no reason why we may not see the establishment of such institutes as we have in mind.

University laboratories have now the burden of finding and training minds that will be capable of handling the great problems that lie so clearly before us. Promising students may be encouraged, not only to become experts in certain fields of our subject but to grasp the major projects of the planning and interpretation of comprehensive research by coöperating groups. Men and women who are now students or beginners will be the ones upon whose shoulders will fall the new mantle of this great responsibility. There is plenty of bright outlook for

students of our subject who can master its many-sidedness and devote themselves to its service. This thought applies also to students in laboratories other than those of plant physiology, for the coöperating groups of which we dream will require minds trained in physics and chemistry, in plant morphology and climatology, in soil science, animal physiology and even astronomy. Indeed, the new plant physiology promises to offer excellent opportunities to the largest number of competent and versatile minds trained primarily in these related subjects. At any rate, the most complex of chemical and physical problems are to be found in the physiological field and the most generally interesting features of non-living masses and particles are, after all, their influences on the vital processes. Having gone so far in understanding the general causal relations of material and energy changes in non-living systems, human intelligence may now make rapid progress toward solving at least some portions of the riddle of life. But the best minds will be needed, and also much of the wealth of knowledge and thought, principle and idea, that have already been accumulated in the realm of the sciences of the non-living.

Perhaps the most pressing need of our science now, as it begins to approach more consciously the causal aspects of plant phenomena, is the need for new or improved instruments and methods, which shall be suited to the routine operations of well-planned physiological experimentation. Consider how inadequate our experimental methods generally are and consider that most outstanding contributions to physiological knowledge involve new technique or improved apparatus and could not be accomplished without it. In experimental procedure the impossible must continually be made possible. We require better means for controlling experimental conditions, better means for measuring the intensities and fluctuations of the processes we are studying and of the influential conditions that affect those processes. Every dynamic characteristic of the organism and every effective dynamic characteristic of the environmental complex needs rational and creative study, by the aid of which may be invented or devised new instruments or methods that will give us the measurements we require, and in suitable terms. How to secure useful measurements of variable conditions and how to integrate our numerical values



into calculated indices that may be compared with other indices secured by direct measurements of the results or products of the processes dealt with—these are our most insistent questions.

These preliminary problems of apparatus and methods are fortunately approachable without very special equipment and without very special arrangements for coöperation, though of course alert mentality and technical skill are demanded. They are questions with which most of us are busied now, and there is much more to be done before we shall be ready for the greater undertakings that lie in the offing. There is no limit to this field of endeavor and no question of the constructive value of and necessity for the results to be secured. We cannot hope that our present experimentation or that of the near future will throw much more than suggestive light on the causal relations that we are hoping to discover eventually, but we can make ready for better planned and interpreted experimentation by groups, when that shall become more feasible. Individual workers can now work out separate tools and methods that will be brought together in the more complex operations of the future. And the new times will bring no end to this kind of individualistic work, for tools and methods will always need to be improved.

In this connection it may be pointed out that, for the present and immediate future, contributions in instrumentation and technique should be regarded as much more important than any general conclusions that may be derived from most of our commonly only abortive attempts to obtain reliable knowledge of causal relations by short-cuts that are not planned to succeed in the broader way. It is not our surmises and opinions or statistical calculations of probable concomitancy or correlation that are of lasting importance. Temporarily interesting and stimulating as these indeed are, they pertain more to the category of amusement and imaginative recreation than to that of really constructive progress. We need to hold in high esteem the devising and adequate testing of new methods and the logical improvement of older methods, for upon such work the future of our science depends.

DESERT LABORATORY, TUCSON,  
SEPTEMBER, 1926.

# INDEX TO AMERICAN BOTANICAL LITERATURE

1923-1926

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